MINERAL AND FIBER DISAPPEARANCE FROM FORAGES ESTIMATED BY IN SITU TECHNIQUES AS INFLUENCED BY DIGESTIVE ENVIRONMENT OF THE RUMINANT

By

STEPHEN M EMANUELE

A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN
PARTIAL FULFILLMENT OF THE REQUIREMENTS
FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1983

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the help and advice of many people. My appreciation is extended to the members of my committee, Drs. D. K. Beede, chairman, C. R. Staples, cochairman and advisor, and to Drs. C. S. Ammerman, J. H. Conrad, J. E. Moore and O. C. Ruelke. Their time and advice have been invaluable. My sincere appreciation to Dr. C. J. Wilcox for statistical advice and Dr. L. C. Peyton for the cannulation of the animals, and Dr. M. A. Delorenzo for his assistance and computer resources. Each of my committee members has influenced my professional development, encouraged imaginative thought and reasoning, and stressed the need for an integrated approach to scientific problem solving.

My deepest gratitude to Joyce Hayen, who analyzed many samples on nights and weekends, going beyond her duties. My appreciation to Rafael Umana, who supervised my final experiment while I attended scientific meetings. Without his help, the final experiment could not have been completed. To my fellow graduate students who helped with sample collection and gave encouragement, I am truly grateful. My sincere appreciation to Dale Hisser, farm supervisor, and his staff, whose sense of humor kept me sane. Deanne Marse's friendship, help and advice has been invaluable. She and my fellow graduate students will be missed greatly.

My wife Patti and children, Christina and James had to bear the burden of this program. They were a source of strength and inspiration, standing by me in good and bad times. They deserve a degree just for putting up with my schedule.

Finally, my deepest appreciaiton to Cathy Freeman for typing this manuscript. I am grateful to Dr. R. P. Hatzke and the Dairy Science Department for providing a graduate assistantship. Without thair financial support, this program would not have been possible. Drs. H. H. Head, W. Harris, Jr. and the other Dairy Science faculty stieulated me to do my bast and raach my potential. Thair advice and intarast in my progrme of study was truly appreciated. My apreciation to Dr. C. R. Staples, who guided and advised me during this progrme. this program

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Abstract of Dissertation Presented to the Graduate School
of the University of Florida in Partial Fulfillment of the
Requirements for the Degree of Doctor of Philosophy

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By

Stephen M. Emanuele

May, 1969

Chairman: David K. Beede
Cochairman: Charles R. Staples
Major Department: Animal Science

Utilization of minerals from forages by ruminants occurs through two processes. First, the mineral must be released from the forage. Then once in the ruminal fluid, the mineral can be absorbed by the animal or used by ruminal microbes. The in situ technique was used in experiment one to estimate the immediately and slowly released fraction for calcium, magnesium, potassium, phosphorus, copper and zinc from six forage species. The forages, alfalfa, rhizoma peanut, dwarf elephantgrass, bermudagrass, bahiagrass and limpograss, were evaluated after ruminal incubation in 17 x 9 cm dacron bags for 2, 4, 6, 12, 18, 24, 30, 36, and 72 h. In experiment two, mobile dacron bags, 8 x 3 cm in size, were used to measure postruminal mineral release from these six forage

species. Experiment three measured the influence of ruminal pH and energy concentration of the diet on mineral release from alfalfa.

In situ ruminal mineral release varied with forage species and mineral. Mean extent of ruminal mineral release across forage species was as follows: potassium (100%) > magnesium (95%) > copper (84%) > phosphorus (80%) > calcium (70%) > zinc (69%). Release of phosphorus, copper, and zinc was lowest for forage species with high NDF concentration. An average of 38% of the calcium was released slowly indicating an association with the cell wall.

The rumen was the major site of release for all six minerals. Maximal digestive tract mineral release from the six forage species was: potassium (100%), magnesium (96%), phosphorus (92%), and calcium (75%). Acid-pepsin incubation increased calcium release an additional 12.5 to 53 percentage units, while lower-tract incubation increased phosphorus release an additional 2.6 to 14.1 percentage units across all forage species.

Energy concentration of the diet and ruminal pH affected ruminal mineral outflow. More of the calcium (55 vs. 40%) and phosphorus (96 vs. 77%) leaving the rumen was in the liquid phase when readily fermentable carbohydrates were in the diet. Apparent absorption of potassium was decreased by acid infusion or supplemental energy.

CHAPTER I
INTRODUCTION

In a basic physiology course, the student is told, that the purpose of science is not to preserve a hypothesis by seeking everything that will support it and setting aside everything that may weaken it. Rather, progress is changing old theories for new ones when appropriate. The beginning premise of these studies was that forages grown under subtropical and tropical environments are high in cell wall concentration, and that in vitro studies and metabolism trials indicate that certain plant fibers can bind minerals. The hypothesis tested was that forages with high cell wall concentration could have reduced mineral release because the cell wall could bind minerals. Factors which influence fiber disappearance may influence mineral release. To test this hypothesis, six forage species and six minerals were chosen. The forages were selected based on their current or potential use in the dairy and beef industry in Florida. The minerals were selected based upon anticipated differences in amounts released in the digestive tract. As nutritionists attempt to improve digestive efficiency, it has become increasingly apparent that nutrients must be provided to the animal in a coordinated fashion. One limiting nutrient such as an amino acid can reduce milk production. Soluble energy must be fed with soluble nitrogen for efficient use of that nitrogen. Minerals from feedstuffs must be characterized to determine their pattern of release to the

animal. Are they being supplied at the "right times", or are some minerals instantly released at feeding such that they may become deficient as fermentation progresses? The total concentration of a mineral in a forage possibly can be divided into three fractions—immediately released, slowly released and unreleased. The unreleased fraction would indicate how much of the total quantity of the mineral would enter the liquid phase of the digestive tract. Once in the liquid phase of the digestive tract, the mineral could be absorbed by the animal, used by rumen microorganisms for maintenance and growth, bind to the plant cell wall, or form insoluble compounds such as copper sulfite. The slowly released fraction could be characterized by a release rate constant and potentially be susceptible to changes in the ruminal environment.

The progression of experiments was, first, characterization of six minerals of six forage species as to the proportion of immediately released, slowly released, and unreleased fractions when incubated in a semi-neutral ruminal environment (pH between 6.2 and 7.0), second, using mobile dacron bags, measurement of extent of mineral release from six forage species consecutively incubated in the rumen, an abomasal-type environment, and intestines of Holstein cows, and third, determination of effect of supplemental, readily fermentable carbohydrates and/or increased acidic conditions in the rumen on apparent ruminal absorption of six minerals from alfalfa hay fed to Holstein dairy cattle.

A better understanding of mineral release from forages, and factors affecting that release, will improve our ability to meet the nutrient needs of ruminant animals in a timely fashion.

CHAPTER II
LITERATURE REVIEW

Introduction

Five classes of feedstuffs are utilized in the formulation of diets for dairy cows. In each diet a roughage, a high protein feedstuff, an energy concentrate, a mineral supplement and a vitamin supplement provide the required nutrients for milk production. Dairy cattle do consume large amounts of grain, but roughage provides the majority of their diet. Because roughages play such a large role in dairy cattle diets, it would behoove the formulator to make maximum use of all its available nutrients. While the availability of minerals in mineral supplements has been studied extensively (Ammerman and Miller, 1978, McDowell et al., 1984, National Research Council, 1978, Thompson, 1978), mineral availability in roughages largely has been neglected.

Before the mineral portion in the plant is released to the animal, mastication, rumination and/or microbial digestion of the feedstuff must occur. Mastication and rumination tear open the leaf and stem. This increases the amount of surface area on which microbial digestion can occur. Neutral detergent soluble material is an estimate of the cell contents of plant material. The cell contents are highly and quickly digestible. A different process of digestion involves the plant cell wall. Extent of cell wall digestion can vary greatly with the forage species, amount consumed, and physical form of the roughage.

The plant cell wall is made of different types of tissue. Mesophyll and phloem are degraded rapidly (Akin and Burdick, 1975). Epidermis cell wall and parenchyma bundle sheaths are degraded slowly. Lignified vascular bundles and sclerenchyma are the tissue types which are mostly undegraded (Akin, 1979). From this, it appears that the microanatomy of the plant may influence the rate and extent of fiber digestion. The distribution of mineral fractions between the cell contents and the cell wall fraction should effect the rate and extent of the release of minerals from a roughage in a ration. Of the minerals affiliated with the cell wall, their release would be related to the rate and extent of fiber digestion in the rumen. Quantification of mineral needs and capacity of roughages to meet the animal's mineral needs are essential. For example, the average availability of calcium in mixed diets fed to dairy cattle ranged from 35 to 38% (National Research Council, 1988). The Agricultural Research Council (1980) used 60% as the estimate of calcium availability.

Mineral Distribution in Forages

The distribution of minerals can be examined from a number of perspectives. Minerals can be partitioned by anatomical location, such as leaf versus stem. Minerals also can be divided by their solubility in different solvents. This method was used by Shockey and Reid (1984) and Todd (1961) to separate magnesium in plants into cell sap, fiber, and chlorophyll fractions. Detergent solutions also have been used to estimate mineral concentration of cell wall and cell content fractions (Kincaid and Cronrath, 1983; McManus et al., 1977).

The mineral concentration of plant cell walls is primarily silicon but also can include metal ions (Jones, 1979). These ions may be bound or complexed with lignin, structural carbohydrates, and silica. Calcium can be bound by pectin, and calcium pectate acts as a cementing agent in the cell wall (Jones, 1979). Studies of the cell wall of the grass Holcus lanatus indicated that pectin and lignin can complex or bind a large portion of calcium and magnesium in this plant (Malloy and Richards, 1971). The main functional groups in the cell wall involved in the binding of metal cations may be carboxyl groups (Malloy and Richards, 1971). The hydroxide group of amorphous silica has a negative charge and may exchange protons for metal ions (Jones, 1979).

At present there are conflicting reports concerning the distribution of the mineral fraction in plants. The laboratory of W. R. McManus holds an opposite view to that of R. J. Harper. McManus et al. (1977) used a scanning electron microscope to obtain information about the physical distribution of mineral material in plant cell walls. These workers examined five tropical grasses, five straws of temperate grasses, three tropical legumes, and three temperate legumes. The amount of mineral material associated with the cell wall varied with forage species. Eighteen to 38% of the ash content of the tropical grasses was associated with the plant cell wall. The percentage of the ash content of the tropical legumes associated with the cell wall was higher, 41 to 53%. This same pattern was observed in the temperate legumes, with 48 to 55% of the ash located in the cell wall fraction. Using energy-dispersive X-ray analysis these workers determined that the major mineral components of alfalfa cell wall were calcium, phosphorus,

sulfur, and potassium, with only small amounts of sodium and chloride. The inorganic material was distributed in sheets, forming a masking layer around potentially disappearable cell wall. The authors felt this cell wall mineral fraction will influence both cell wall disappearance and rate of turnover of minerals.

In a follow-up study (McManus et al., 1979), these workers examined biocrystallization on the plant cell wall fractions. Neutral detergent fiber (NDF) and acid detergent fiber (ADF) fractions had different mineral concentrations. The ADF fraction was primarily silicon with small amounts of potassium and calcium. The NDF fraction contained silicon, calcium, phosphorus, and sulfur as the major ions, but also contained magnesium and potassium. By comparing the difference in mineral profiles of the NDF and the ADF fractions, the authors suggested that calcium and phosphorus were associated primarily with the hemicellulose fraction of the cell wall. Nawawar, Harper et al. (1982) felt these mineralized sheets over the cell walls were formed by dust or contamination of the roughage samples, chemical extraction with neutral detergent solution, and ashing at 500°C. This could have led to the formation of calcium-phosphate crystals. Regardless of the form of calcium and phosphorus, others agree that they are bound to hemicellulose and lignin (Butler and Jones, 1973). The attachment of calcium onto the phosphoryl, hydroxyl, and carboxyl groups of hemicellulose has been demonstrated (Malloy and Richards, 1971).

Release of Minerals from Forages

The rate at which minerals are solubilized or released from roughages may depend on the mineral, plant species, and the rate and extent of fiber disappearance. Those minerals associated with the cell contents should be released quickly, while those associated primarily with the cell wall should be released the slowest. A general ranking of minerals, from fast to slow, according to their release from forages was the following: sodium, magnesium, sulfur, potassium, nitrogen, copper, calcium, and zinc (Playne et al., 1978, Reake et al., 1983). Because tropical forages generally are higher in NDF content than are temperate forages, overall mineral release from tropical forages may be lower. This appears to be the case based on two studies. After 48 h of ruminal fermentation, only 20 to 50% of the nitrogen, calcium, and phosphorus was released from the tropical forages speargrass and Stylosanthes humilus (Playne et al., 1978). This is less than the 55 to 85% release for these same minerals from the temperate forages, alfalfa or ryegrass silage (Reake et al., 1983). Both of these trials used the in situ technique and similar ruminal incubation times.

Little work has been undertaken to determine the distribution and rate of release of minerals from temperate and tropical roughages in the United States. Most of the work performed in this area has been in Australia. Kincaid and Cronrath (1983) at Washington State University stated that data on the bioavailability of minerals from forages are sparse for cattle. The situation has progressed little since their study. They analyzed alfalfa hay and grass haylage for mineral concentration and distribution. They reported that major minerals associated

with NDF residue were calcium and phosphorus, with small amounts of potassium and magnesium. Iron was the predominant microelement found in the cell wall. These workers suggested that minerals associated with the fiber fraction of the forage may be released slowly and be less available to cattle than minerals associated with the cell contents of the plants. Little is known about the effect of roughage quality or lignification on release and availability of minerals from roughages for cattle in the United States. To truly understand the value of our forages in supplying the mineral requirements of our cattle, the study by Kincaid and Cronrath (1983) must be expanded.

Bioavailability of Minerals

It is recognized that the total content of a mineral in a complete ration has little significance. It must be qualified by an availability factor. Availability has been defined as percent digestion, percent absorbed, or percent net retention (Pesler, 1972). Regardless of how one defines availability, a mineral must be in a form that can be solubilized in the rumen. If it is not in a form that can be absorbed, it cannot be transported in the body. Minerals in forages need to be released from the plant material and become soluble in the rumen or small intestine. This solubilization process can be just the release of the mineral from the forage cell contents into the digestive tract, or through microbial activity on the forage.

When considering the mineral concentration of feeds, we must consider the following three mineral fractions: 1) rapidly released and potentially available, 2) slowly released and potentially available, and 3) unreleased and unavailable.

The sum of fractions one and two can be considered the potential availability of a mineral to the animal. Availability and absorption may not be the same. A mineral may be released from a roughage but not absorbed by the animal. This may occur if the released mineral forms an unavailable complex with other ions in solution. Absorption also may be hindered by unfavorable conditions in the digestive tract. One example would be the Na:K ratio on magnesium absorption. Absorption may also be regulated by the mineral status of the animal. This is true of calcium, where release from a roughage can be fairly stable for that roughage in a specific ruminal environment, but absorption can vary depending on the nutritional need of the animal (Miller, 1983). This allows the animal to tolerate a wide range of calcium in the diet. A lack of knowledge on the availability of calcium in feeds is a major reason for differences in opinion on the amount of calcium recommended in the diet (Hansard et al., 1957). Ward et al. (1979) recommended twice the level of calcium in the diet for lactating dairy cows as compared with the National Research Council recommendation (1978). This was based on lower availability of calcium in alfalfa as compared with other forages.

Calcium

The dietary requirements for calcium are determined by a factorial method (National Research Council, 1988). Most researchers agree on the amount of available calcium needed by the animal (Miller, 1983). Disagreement exists on how much of the calcium in feedstuffs is truly available. The Agricultural Research Council (1980) estimated availability of forage calcium at 68%. The National Research Council (1978) used an estimate of 45% for calcium availability. Recently it has been

suggested that this estimate is too high. Ward et al (1979) and Ward and Habers (1982) found that calcium in alfalfa was only 50% as available as calcium from inorganic sources. This led them to recommend twice the National Research Council recommendation for calcium during early lactation. Recently, the National Research Council lowered the estimate of calcium availability to 38% (National Research Council, 1988). A reoccurring problem in examining the literature is the different definitions of availability. Solubility and availability are synonymous in some cases. Sometimes availability refers to absorption or retention. For example, calcium carbonate is stated to have a solubility of 40 to 51% and an availability of 100% (Thompson, 1978). This means that one-half of the calcium in calcium carbonate is soluble, and 100% of this soluble calcium can be absorbed. It may not, however, all be absorbed.

Calcium absorption occurs primarily in the small intestine, by both active and passive processes (Hillar, 1983). The main homeostatic control mechanisms for calcium status are in variable rates of 1) absorption from the gut, and 2) deposition and resorption from the bones. The amount of available calcium absorbed by the lactating cow will depend on stage of production and the amount of available calcium in the diet. Homeostatic control of calcium absorption allows the ruminant to tolerate a wide range of calcium:phosphorus ratios (Hansard et al., 1957). Calcium distribution in plants has been examined by electron microscopy and by fractioning the calcium into different plant fractions (Jones, 1978; McManus et al., 1977; McManus et al., 1979). They observed that calcium in alfalfa was associated with sclerenchyma

tissue in the stem and parenchyma tissue in the leaf. They reported that calcium is the main cation in the cell wall and that its release may be limited by silica and lignin in the plant. If substantial calcium is associated with the cell wall, release may depend on whether calcium is bound to hemicellulose, cellulose, or lignin.

Release then will have to be predicted using an equation which accounts for three sources of calcium in feeds. Availability would be dependent on the proportion of calcium in the cell contents and the rate and extent of release of cell wall calcium. It would seem that such an equation would have to include an estimate for the effect of lignification on plant cell wall digestibility.

Phosphorus

The National Research Council (1988) calculated the phosphorus requirement of dairy cattle based on an availability of 90% for young calves and 50% for older cattle. The phosphorus requirement for milk production is based on the phosphorus concentration of milk and a 50% phosphorus availability. Some factors that may influence the utilization of phosphorus are type of ration, chemical form of the mineral, age of the animal, sex of the animal, plane of nutrition, calcium:phosphorus ratio in the diet, plus other factors (Thompson, 1978). All these factors make it difficult to determine phosphorus absorption from released phosphorus in a ration. As with calcium, the majority of research on availability has been performed with mineral supplements. Research on phosphorus release from forages is needed. Recent data indicates the release of phosphorus from forages is affected by forage species (Playne et al., 1972, Playne et al., 1978, Meake et al., 1983)

In situ phosphorus release from alfalfa was 83%, but in situ release from speargrass and Stylosanthes humilus was 30 and 37%, respectively (Playne et al., 1978). There appears to be a difference between temperate and tropical grass species. Phosphorus release in ryegrass silage was estimated from in situ data to be 66 to 73%, depending upon rate of passage (Rooke et al., 1983).

Phytate Phosphorus

Two-thirds of the phorphorus in most grains is in phytate (Nelsen et al., 1968). The inositol ring in phytate must be hydrolyzed by the enzyme phytase before phosphorus can be absorbed. Though grains contain phytate phosphorus, forages contain little phytate. Nelson et al. (1976) reported that fresh alfalfa, bermudagrass, bromegrass, orchardgrass and fescue plus bermudagrass, bromegrass, and fescue hays did not contain any phytate phosphorus. Clark et al. (1986) reported that phytate phosphorus intake was totally dependent on grain and grain byproduct consumption by lactating cows. Cows were fed a corn silage based total mixed ration with a 50:50 forage to concentrate ratio. Daily phytate phosphorus intake averaged 38.3 and 46.2 g/cow during wk 4 and 10 of lactation. The corn silage portion of the ration provided 1 g/d phytate phosphorus. Hydrolysis of natural phytate phosphorus is high. Hydrolysis of phytate was 100% by steers (Nelson et al., 1976), 58 to 99% by lactating cows (Clark et al., 1986), 100% by sheep (Reid et al., 1947), and 92% by sheep fed a semi-purified diet (Tillman and Brethour, 1958). It can be concluded that ruminants can potentially utilize almost all of the phytate phosphorus in feeds. There probably

is no reason to discount the phytate phosphorus found in grains and forages fed to ruminants

Calcium and Phosphorus and the Lactating Cow

R. L. Horst (1986) reviewed the scientific literature on calcium and phosphorus metabolism in the lactating cow. He concluded there was still a lack of knowledge on the availability of calcium and phosphorus in common ruminant feedstuffs. He felt information was sparse on the effects of different physiological states on calcium and phosphorus availability. Horst concluded his review with the following statement: "Recent estimations of dietary calcium availability during lactation led support to increase the recommended calcium concentration in the diet of high-producing dairy cows from 65 to 75%, particularly in early lactation." He calculated total dietary calcium required to maintain calcium balance during lactation. Using the assumption that dietary calcium absorption was 38% and dietary phosphorus absorption was 40 to 50%, he suggested at least a 10 to 20% increase in both dietary calcium and phosphorus concentrations for dairy cows as recommended by the National Research Council (1978). These absorption values for calcium are from Hansard et al. (1957), and for phosphorus, from studies with sheep (Braithwaite, 1983). The National Research Council (1988) has increased the calcium and phosphorus recommendations for lactating cows. Their recommendations for cows in early lactation are .77% calcium and .48% phosphorus. These recommendations agree with Horst (1986). It is not in the scope of this review to criticize these estimates. Rather, it is to point out the small database from which these calculations were based. There is a need to compare different forages or feedstuffs under

similar conditions in the same animal. One question is unanswered still. Is calcium and phosphorus from roughages less available than from inorganic sources? If this were true, then some way is needed to discount the calcium and phosphorus concentration from forages. A step toward estimating mineral availability is to first determine the amounts potentially released by the forage to the digestive environment. The in situ technique or in vitro technique could be used to estimate mineral release. These techniques could be used to rank forages according to mineral release coefficients and determine if cell wall content of the forage affects calcium and phosphorus release in dairy cattle.

Magnesium

The adult animal body contains about .05% magnesium by weight. Retention of available magnesium by dairy cattle is related to body needs. Excess magnesium in the diet is excreted in the urine. Many factors influence magnesium availability. The major factors are composition of the diet, magnesium status of the animal, mineral interactions, chelates in natural feedstuffs, age and genetic differences in animals, amount of fertilization to the growing crop, stage of maturity of the harvested feedstuff, and growing season of the forage (Thompson, 1979). Apparent absorption of magnesium in forages ranged from 10 to 25% (Lomba et al., 1968, Rook and Storry, 1962). These data were collected in balance trials with sheep and cattle. An in situ study found over 50% of the magnesium in grass silage was released in the rumen, and that its rate of release was rapid (Roche et al., 1983). A portion of the soluble magnesium can form an insoluble complex in the rumen and small intestines after release from the forage, which may

account for the difference between solubility and absorption. Possible complexing factors are the various fiber fractions. It has been shown in vitro that lignin, hemicellulose, pectin, and cellulose can bind magnesium and trace minerals in solution at a typical ruminal pH (Mod et al., 1982; Reck and Storry, 1982). Sixty-seven to 100% of the bound magnesium was released by subjecting hemicellulose to changes in pH or treatment with pepsin or trypsin (Molley and Richards, 1971). Further work should determine if binding of magnesium takes place in vivo and if this bound magnesium is released further down the digestive tract.

Magnesium content of forages decreases with maturity. Magnesium concentration of tropical grasses decreased 12 to 33% as maturity increased from 4 to 12 wk of age (Gomide et al., 1969, Gomide, 1978). Magnesium concentration can decrease as much as 57% in bermudagrass hay with increasing maturity (Ammerman et al., 1982). Older forages may not supply adequate magnesium because of a decrease in magnesium concentration. Less mature forages may not supply adequate magnesium for animal metabolism, because magnesium available is lower than that of more mature forage (Heyland and Grunes, 1979).

The rumen is the major site of magnesium absorption in sheep (Tomas and Potter, 1976; Martens et al., 1978) and cattle (Teller and Gadeau, 1987). In vitro studies on magnesium absorption using isolated ruminal epithelium from sheep have shown that absorption occurs by an active transport mechanism and the mechanism is not saturated until magnesium concentrations reach 5 millimoles (Martens et al., 1978). Brown et al. (1978) obtained similar results with isolated rumen pouches of sheep. During five experiments with one sheep, they concluded

magnesium transport was an active process because, the potential difference between the pouch solution and venous blood was a positive 25 millivolts. Magnesium influx into the blood would occur against an electro-chemical gradient. Martens et al. (1979) examined the mechanism of magnesium absorption with isolated sheep rumen epithelium incubated in Ussing chambers. Their results indicated that magnesium transport has features characteristic of other active transport processes. First, net transepithelial efflux from the lumen to the blood in the absence of a concentration gradient and against a positive transmural potential difference (blood side positive) can occur. Second, magnesium transport seemed to require a functional Na/K ATP pump. Magnesium transport was inhibited markedly by the addition of ouabain. Ouabain inhibits Na/K ATPases. Third, magnesium transport across the ruminal epithelium from the lumen to blood was saturable. Saturation was reached when magnesium concentration in the bathing solutions was 5 millimoles. Fourth, the rate of magnesium transport was temperature sensitive. Lowering the temperature of the bathing solutions decreased magnesium transport. Fifth, the amount of magnesium transported across the epithelial membrane was dependent upon the duration of the experiment, supply of metabolizable energy, and on a viable epithelial structure.

If magnesium transport across the ruminal wall is a saturable, energy-dependent active process, then main factors controlling magnesium absorption in the rumen would be magnesium concentration in the liquid phase of the digesta, dietary constituents such as potassium, ammonia, and fatty acids, and ruminal conditions such as pH, osmolality, liquid dilution rate, and volatile fatty acid production. Recently, a number

of experiments were completed which helped quantify the effects of these factors (Martens et al , 1987, Gaebel et al , 1987; Merteus and Stossel, 1988, Johnson et al , 1988, Grace et al , 1988)

Martens et al (1987) attempted to separate the effect of potential difference (PD) across the ruminal epithelium from the effect of potassium on magnesium transport Isolated mucosal strips of sheep rumen were placed into a Ussing chamber Sheep epithelium was incubated with three different buffers The control buffer was a choline chloride buffer with a Na:K ratio of 4:1 Other buffers were a high potassium buffer with a Na:K ratio of 2:7 and a high sodium buffer with a Na:K ratio of 17:1 Flux studies were performed with the high potassium buffer under open-circuit conditions (K dependent PD) or where the PD was set at 0 millivolts Flux studies performed with the control buffer were done at PD of 0 millivolts or set to the PD of the potassium buffer under open circuit conditions At high mucosal potassium concentrations, transport of magnesium from the mucosal side to the serosal side of the epithelium was decreased and flow of magnesium from the serosal side to the mucosal side increased These changes in flux were caused by an increase in PD across the membrane When potassium concentration was high and the PD set to 0 millivolts, magnesium transport was similar to the control with PD at 0 millivolts. When the PD was set at 25 millivolts (open circuit level), magnesium flux in the control buffer was the same as the high potassium buffer with PD of 25 millivolts The authors concluded that, at high mucosal potassium concentrations, the active transport of magnesium was disturbed by a depolarization of the membrane, and passive flow of magnesium from the serosal to mucosal side

of the membrane was increased. The result was a net decrease in magnesium uptake.

If the above mechanism is correct, then diet and ruminal pH and osmolarity should influence magnesium absorption from the rumen. Gaebel et al (1987) studied the effect of diet, ruminal pH, and osmolarity on magnesium absorption from the rumen of sheep. Diets were given sequentially and were 1) hay only, 2) 36% hay 64% concentrate, 3) 10% hay 90% concentrate, and 4) hay only again. The temporarily isolated reticulorumen technique (Care et al., 1984) was used to measure absorption of sodium, chlorine, and magnesium in sheep. This technique requires emptying of the rumen, washing the rumen six to eight times with a buffer, collection of saliva with a saliva collector inserted into the distal part of the esophegus, and placing a catheter into the reticuloomasal orifice to deliver saliva to the omasum and seal the reticuloomasal orifice. Three solutions of different pH and osmolarity were used to alter ruminal conditions on each diet. They were 1) control solution (pH 6.79, 315 mosmol/L), 2) hypertonic solution (pH 6.77, 422 mosmol/L), and 3) acidic solution (pH 4.79, 318 mosmol/L). Chromium EDTA solution was used to validate the technique. Recovery of chromium ranged from 92 to 99% of initial dose, which indicated chromium was mostly nonabsorbable in the EDTA complex.

Diet did influence the amount of surface area available for mineral absorption. Ruminal papillae biopsies from three sites revealed that surface area of the papillae at all sites increased almost 200% in sheep consuming the 64% concentrate diet. Feeding a 90% concentrate diet increased the surface area almost 400%. These changes were fully

reversible when the animals were returned to the hay only diet. Sheep were fed the dietary treatments 8 wk, so the exact time necessary for these morphological changes to occur is not clear.

Elevation of osmotic pressure from 315 to 422 mosmol/L caused a net secretion of water into the rumen across all diets. Net absorption of sodium, chloride, and magnesium and PD was not affected by increased osmotic pressure ($P > .05$). Changing the diet influenced magnesium absorption. Net absorption of magnesium was decreased 53, 91, and 72% by sheep receiving the 0%, 64% and 90% concentrate diets, respectively. Sodium and chloride net absorption rates were reduced, but to a lesser degree as intake of concentrate increased. Interestingly, the decrease in the net ion absorption was accompanied by a decrease in the PD when the acidic buffer solution was used. The drop in PD was smaller with concentrate feeding, but absorption rates of ions on all treatments was lower than control levels ($P < .05$) when the pH was lowered.

The reduced net absorption of sodium and magnesium at lowered ruminal pH could be caused by a disruption in active transport or increased passive diffusion into the rumen. This study does support the in vitro Ussing chamber experiment of Martens et al. (1997). Based on these studies, it appears that high potassium concentrations in the rumen or very acidic conditions alter the PD of the ruminal epithelium, and this change can disrupt Na/K ATPase activity. The result in either situation (high potassium or low pH) is a reduction in active transport of magnesium.

It is important to remember that both Martens et al. (1997) and Gaebel et al. (1987) used techniques which may not simulate actual

conditions in the rumen under normal feeding regimes. Johnson et al (1988) studied magnesium metabolism in lactating Jersey cows fed frozen grass or hay plus concentrates. Concentrates were a mixture of barley meal and dried beet pulp. Daily DM intake of the hay plus concentrates was 8.1 to 8.7 kg, and 9.5 to 8.7 kg of the grass diet. Cows were fed hay plus concentrates for 3 d, then changed to frozen grass. This abrupt dietary change altered the ruminal environment. Ruminal pH decreased from 6.69 to 5.85 in experiment 1, and 6.60 to 6.27 in experiment 2 (repeat of experiment 1). Mean PD between ruminal contents and blood (positive) increased from 26 millivolts to 38 millivolts when diet changed from hay plus concentrates to frozen grass ($P < .001$). Ruminal potassium concentration was increased when sheep were changed to the frozen grass diet from the hay plus concentrates diet. This resulted in a decreased Na:K ratio. All these intraruminal changes should decrease magnesium absorption based on studies with sheep (Martens et al., 1987; Gaebel et al., 1987). Although Johnson et al. (1988) did not measure magnesium absorption directly, plasma magnesium concentration and urinary excretion of magnesium was decreased by cows receiving the grass diet. This was accepted as an indication of decreased magnesium absorption from the rumen.

Grace et al. (1988) reported similar results with sheep. Three sheep were fed dried grass-maize pellets and three others fed frozen grass for 25 d. For the next 14 d, KCl was infused continuously into the rumen. During the 25 d control period, potassium intake was 22 g/d. This was increased to 50 g/d by potassium chloride infusion. The increased potassium load decreased plasma magnesium concentrations,

increased fecal excretion of magnesium, and decreased urinary magnesium output of all sheep regardless of diet. In a second experiment, these authors used the temporarily isolated rumen technique to study rate of magnesium absorption at three Na:K ratios. These were 1) low potassium buffer with Na:K ratio of 10:3, 2) medium potassium buffer with Na:K ratio of 4:3, and 3) high potassium buffer with Na:K ratio of 1:2. Magnesium absorption decreased from 23%/h when the Na:K ratio was 10:3, to 9.1%/h when the Na:K ratio was 1:2 ($P<.001$). Differences between sheep in their ability to absorb magnesium were greater than the effects of increasing potassium concentration. They suggested that this large variation among animals may reflect differences in the absorptive area of the rumen papillae. Still, their in vivo experiment with sheep supported the results obtained with the Ussing chamber (Martens et al., 1987).

Does large variation among sheep in their ability to absorb magnesium exist for cattle, and does stage of lactation affect magnesium absorption? Greene et al. (1986) performed an experiment to determine if breed or DM intake influenced magnesium availability to dry, non-pregnant, mature cows. Sixty cows of five breeds (Angus, Brahman, Hereford, Holstein and Jersey) and their F1 crosses were used in a metabolism study. The total number of breed groups was 15, five purebred and ten crossbred groups. Treatments were arranged in a 4 x 4 Latin square design. Treatments were 50, 83, 117, and 150% of each animal's estimated daily maintenance energy requirement. Cows were fed a cottonseed hull-based diet which contained 70% hulls, 15% cottonseed meal, 10% ground milo, and 5% molasses plus minerals and vitamins. No

significant interactions between breed and magnesium intake (g/d) were observed. As feed intake increased from 50 to 150% of maintenance, fecal magnesium excretion increased ($P < .05$). Apparent magnesium absorption increased from 13 to 43% of magnesium intake as feed intake increased from 50 to 150% of maintenance ($P < .05$). This increase in percent magnesium absorption may be associated with an increase in energy intake.

Breeds differed in magnesium intake, fecal magnesium excretion, and apparent absorption ($P < .05$). Hereford, Holstein, and Jersey breeds excreted more magnesium in the feces and absorbed less magnesium than Angus and Brahman breeds. Magnesium absorption was not different for Holsteins and Jersey breeds ($P > .05$). Brahman and Brahman-cross cows tended to absorb more magnesium than did any other breed group. These breed differences were large. Brahman cows apparently absorbed 41% of dietary magnesium, whereas Holstein and Jersey cows absorbed 25 and 17%, respectively. Predicted true magnesium absorption was 63% summarized over breed groups. Brahman cows had higher true magnesium absorption coefficients, 77%, than Holstein or Jersey cows, 41 and 42%, respectively.

The physiological state of an animal can affect the amount of magnesium absorbed from the rumen. Teller and Godeau (1987) fed 67 different diets to three dry and one lactating Friesian cow fitted with a re-entrant cannula in the proximal duodenum. Type of diet had no effect on apparent magnesium absorption, but values obtained with the lactating cow were lower than the non-lactating cows. The amount of magnesium absorbed (g/d) was related to magnesium intake (g/d). The

equation describing this relationship pooled across all animals and diets was

$$\text{Mg absorbed (g/d)} = -5.89 + .9(\text{Mg intake, g/d})$$

$$n = 67 \text{ and } r = .85$$

Eighty percent of magnesium intake was absorbed.

In summary, the major site of magnesium absorption is the rumen. Magnesium absorption can occur by two processes. The major route is transcellular Na/K ATPase-mediated active transport with passive transport between cells also possible. Low ruminal pH or high ruminal potassium concentrations disrupt the PD of the ruminal epithelial membrane and decrease active magnesium transport. Energy intake can have a positive affect on magnesium absorption by increasing the surface area of the rumen papillae due to increased butyric and propionic acid production (Gaebel et al., 1987). This positive effect may be negated by the drop in ruminal pH which accompanies increased energy intake. Given our current understanding of magnesium metabolism, maximal magnesium absorption should occur on diets of high energy concentration that are buffered with a neutralizing or buffering agent such as sodium bicarbonate. Sodium bicarbonate would keep the Na:K ratio high and ruminal pH higher and more consistent. The high dietary energy content would increase the absorptive surface area and blood flow to the rumen. All these physiological conditions should be optimal for magnesium absorption from the rumen.

Additional factors affecting magnesium availability to ruminants. Some of the interrelationships of potassium, magnesium and nitrogen in ruminants have been summarized by Fontenot et al. (1973).

1. Feeding nitrogen at high concentrations does not appear to influence magnesium absorption, regardless of the nitrogen form.
2. High concentrations of dietary nitrogen result in increased urinary magnesium excretion and decreased magnesium retention.
3. High concentrations of dietary nitrogen do not appear to decrease magnesium utilization more than high levels of potassium alone.

These often contradictory conclusions are based upon the results of Meera et al. (1972). They examined the effects of dietary crude protein (10 or 32% of diet DM) and nonprotein nitrogen (0 or 35% of total nitrogen) on magnesium utilization by wether lambs. Any attempt (Fontenot et al., 1973) to minimize the effect of nitrogen on magnesium utilization ignores earlier studies with lactating cows and goats. Lactating cows were fed forage with differing nitrogen content, increased by fertilization (Kemp et al., 1961). High nitrogen fertilization increased nitrogen, sodium, calcium and magnesium concentrations in the forage. Increasing forage nitrogen from 2 to 4% decreased apparent magnesium availability from 22 to 10%. High nitrogen fertilization of orchardgrass hay decreased apparent magnesium availability from 34 to 12% in lactating goats (Daniel et al., 1979). Apparent magnesium availability was 18 to 24% when sheep were fed forage with a nitrogen concentration of 2 to 2.6%. When forage nitrogen was increased to 3.7 to 4.4%, apparent magnesium availability was 11 to 16% in the same sheep (Stillings et al., 1964). A confounding factor is

that when forage nitrogen percentage is increased, forage potassium concentrations also are increased sometimes (Barta, 1973). This has led some to claim the effect of nitrogen concentration on magnesium utilization is really a potassium effect (Fontenot, 1973). Others have concluded that ruminal ammonia levels affect magnesium absorption only in situations where a rapid rise of ammonia exists, with eventual adaptation by the rumen mucosa (Mayland and Grunes, 1979). Recently, Tellar and Godeau (1987) suggested that the effect of ruminal ammonia on magnesium utilization was more negative in diets containing natural protein than urea.

Given these differing views on the possible effect of nitrogen on magnesium utilization, it is not surprising that scientific investigation switched to other areas such as potassium. To test these views, an experiment should be designed such that potassium concentrations are held constant and the Na:K ratio equal on all treatments. Nitrogen concentrations should be varied using natural protein and/or urea. Treatments should be isocaloric but differ in the amount of rapidly degradable protein. This should result in different ruminal ammonia concentrations, while potassium and energy concentrations in the diet remain constant.

Sodium may have a positive effect on magnesium absorption in the rumen. If magnesium absorption from the rumen is an active process involving a Na/K ATPase, then ruminal sodium concentration may influence magnesium absorption. O'Connor (1987) determined the effects of increased dietary concentrations of potassium, magnesium, and sodium on apparent disappearance of magnesium from the rumen of lactating cows

When magnesium concentration in diet DM was increased from .20 to .40%, magnesium concentration in the ruminal liquid, ruminal solid phase, and liquid phase outflow (g/d) was increased. The ratio of sodium to potassium was altered by changing potassium concentration (.97 or 1.65%) and sodium concentration (.22 or .60%). Alteration in the Na:K ratio did not appear to affect magnesium disappearance from the rumen. A sodium by magnesium interaction on magnesium outflow from the rumen was detected ($P < .10$). Magnesium liquid phase outflow was not affected by higher sodium concentrations (.60%) when dietary magnesium was .20%, but outflow from the rumen was decreased 21% when the .40% magnesium and .60% sodium diet was fed. O'Conner suggested that this reduced magnesium outflow from the rumen was the result of enhanced magnesium absorption due to higher ruminal sodium concentration. This was certainly possible as magnesium concentration of the solid phase of the ruminal digesta was not affected by sodium concentration, nor was ruminal liquid outflow (L/d) affected by dietary sodium concentration. These data suggest that sodium concentration in the liquid phase of the ruminal digesta could effect apparent magnesium disappearance.

Another factor affecting magnesium availability is readily fermentable carbohydrate concentration of the diet. Madsen et al. (1976) supplemented a red clover and orchardgrass diet with three amounts of glucose in a sheep study. Apparent magnesium absorption was increased linearly from 27% at zero glucose supplementation to greater than 34% when 950 kcal/d were coming from glucose ($P < .01$). Varying the amounts of supplemental glucose did not influence apparent absorption of calcium, potassium or phosphorus. Supplementation of forage diets with

sucrose increased apparent magnesium availability to sheep (House and Mayland, 1976). Readily fermentable carbohydrates in the diet have been shown to reduce the incidence of grass tetany and are considered important to keep the ratio of nitrogen to total water-soluble carbohydrates low. A high ratio of nitrogen to total water-soluble carbohydrates has been linked to the incidence of grass tetany (Mayland and Grunes, 1979).

In addition to carbohydrates, other energy sources have an effect on magnesium utilization. Long chain fatty acids (LCFA) can have a negative effect on magnesium availability. There is a negative relationship between forage magnesium availability and LCFA content of the diet in lactating cows (Mayland and Grunes, 1979). Wilson et al. (1969) supplemented the diet of lactating cows grazing perennial ryegrass with 454 g of starch or 225 ml peanut oil or no supplement. Cows receiving peanut oil had depressed blood plasma magnesium concentrations, and cows receiving starch had increased blood plasma magnesium concentrations compared to control cows. These authors concluded that the formation of magnesium soaps in the rumen reduced forage magnesium availability. The practice of including LCFA in dairy rations has been increasing. Dairymen are using whole cottonseeds, whole soybeans, animal fat and oils to increase the energy density of cattle diets. Supplementing these types of diets with additional magnesium to offset the possible reduction in magnesium absorption may be profitable. Research needs to quantify the magnitude of the negative effect of LCFA on magnesium absorption.

Potassium

Forages generally contain more potassium than required by dairy cattle. Diets high in concentrates, as fed in early lactation, may not meet adequately the requirement. In general, potassium is very soluble in the rumen and rapidly absorbed by cattle. Potassium from grasses, legumes and inorganic sources is readily available (Gomide et al., 1969, Gomide, 1978). After 12 h of incubation with ruminal microorganisms, 75 to 85% of the potassium in forages was released (Playne et al., 1978). Rooke and coworkers observed 67% of the potassium to be released from forages after 2 h of in vitro incubation (Rooke et al., 1983). This indicates that potassium is not associated primarily with the cell wall. If this is a correct assumption, apparent potassium availability could be predicted from a linear regression equation.

One such equation would be $y = a + b(x)$, where b = absorbable potassium in cell contents, x = total potassium in forage. If potassium has the same absorption as the cell contents, the equation could be written as $y = a + 0.98$ times total potassium in forage, where the amount of potassium available as a percent of absorption would be represented by y and a = endogenous fecal potassium excretion. This equation would be correct as long as there is a linear relationship between the absorbable amount of potassium in a feed and the amount of total potassium in the feed. It must be determined if this type of relationship is true for potassium. A similar relationship may exist for magnesium and sodium.

Copper

With the exception of phosphorus, deficiency of copper is the most widespread mineral deficiency for grazing livestock in the tropics

(McDowell et al., 1984). Copper deficiencies in ruminents occur mainly under grazing conditions. Four reasons for copper deficiency are 1) high levels of dietary molybdenum, > 20 ppm in feed, 2) low copper but significant amounts of molybdenum in ration, 3) deficient dietary copper, < 5 ppm, and 4) adequate copper and low molybdenum with high levels of sulfide in the rumen. Due to the interaction of copper with molybdenum and sulfur, determination of copper in the diet or pasture has limited diagnostic value. Pastures may test adequate in copper, yet deficiencies can occur because of unusual molybdenum and sulfur concentrations. It is true that copper must be released from the forage in order to react with molybdenum and sulfur. When copper is adequate in the pasture, and molybdenum and sulfur concentrations are not excessive, copper deficiency can occur still. Two possible explanations for this occurrence have been proposed.

Copper can become unavailable to the animal after it is released from the forage or feed. Suttle et al. (1984) suggested that ingestion of soil high in iron impairs copper absorption when sulfur content of the diet was 41%. An alternative explanation could be that the copper in the forage is bound to cell wall constituents and not available to the animal. Whitehead et al. (1985) investigated the distribution of mineral elements in forages. Perennial ryegrass, tall fescue, white clover, and lucerne were evaluated. The plant cell wall was seperated by mechanical disintegration followed by washing with chloroform, acetone and ether. The proportion of copper associated with the plant cell wall was 18 to 44%. If copper in the cell wall is bound to lignin, it may not be available to the animal. Under these conditions, copper

deficiency could occur on pastures adequate in copper. It must be determined if copper in tropical and subtropical forages is found primarily in the plant cell wall. The availability of the copper found in the cell wall fraction also must be quantified.

Zinc

Zinc is involved in nucleic acid and protein metabolism. It serves as part of the structure of metalloenzymes and as a cofactor for zinc-dependent enzymes. The utilization of amino acids in the synthesis of protein is impaired during zinc deficiency. Zinc requirements of ruminants vary with the chemical form of the element (Ammerman and Miller, 1978). The zinc recommendation for dairy cattle is 40 ppm (National Research Council, 1988).

A zinc deficiency for grazing livestock once was thought unlikely under practical conditions. McDowell et al. (1984) reported cases of zinc deficiency in grazing cattle occurred in 16 Latin American countries and three each in Europe, South Africa, and the US. McDowell et al. (1984) recommended adding 20 to 30 ppm supplemental zinc to ruminant diets under tropical conditions. The exact cause of zinc deficiency under grazing conditions is not clear. In most cases, the concentration of zinc in the plant is below 40 ppm. In some cases, zinc deficiency has been reported when the forage contained greater than 40 ppm zinc. This may be caused by available zinc forming complexes with calcium, copper or other dietary components (Butler and Jones, 1973). Alternately, zinc in the plant may not be released because it is found in lignified cell walls. Butler and Jones (1973) reported that studies performed with radioactive zinc indicate that plants remove

large amounts of zinc from the soil by binding to cation exchange sites in the plant cell wall. Additional support comes from Turner (1970) who observed a high distribution of Zn^{65} in the cell wall fraction of Festuca prepared by differential centrifugation. Whitehead et al. (1985) reported 24 to 78% of the zinc in temperate forages was located in the cell wall of the plants. Future work should confirm if this is the situation in tropical and subtropical forages. If most of the zinc in plants is associated with the cell wall, availability of this zinc needs to be determined.

Lactational Responses to Dietary Sodium, Potassium and Magnesium

It is important to quantify the contribution of forages toward supplying the lactating cow's requirement for sodium, potassium and magnesium. This has taken on added significance because some recent trials have reported responses in milk production when dietary sodium, potassium and magnesium concentrations in the diet were increased above recommended concentrations (National Research Council, 1978). Schneider et al. (1986) evaluated the effect of environment (shade vs. no shade) on lactational responses of dairy cows. Sodium concentration in the diet was .18, .55, or .86% of DM. Potassium concentration in the diet was 1.3 or 1.8% of DM. The sodium source was either sodium bicarbonate or sodium chloride. This experiment was conducted during the summer in Florida. Cows were experiencing heat stress as indicated by elevated respiration rates and rectal temperatures. Four percent fat-corrected milk (FCM) yield increased 9.8% when sodium concentration was increased to .55% of DM with sodium bicarbonate. Milk yield (4% FCM) did not change when sodium concentration was increased to .88% of diet DM. Milk

yield was increased when potassium concentration increased from 1.3 to 1.5% of diet DM, but this response was due to increased feed intake at the higher potassium concentration. Schneider et al. (1984) also reported increased milk production when dietary potassium was increased from 1.0 to 1.5% of DM. O'Connor et al. (1988) did not observe increases in milk production when dietary sodium was increased from .24 to .62%, nor did she observe any effect of increased dietary potassium (1.14 or 1.59%) on milk production of Holstein cows during the winter. O'Connor et al. (1988) reported that 4% FCM was increased 9.8% when magnesium concentration of diet increased from .24 to .48% of DM. Toh et al. (1985) also reported increased milk production when dietary magnesium was increased to .44%.

In Situ Technique

The suspension of feedstuffs in the rumen using the in situ bag technique allows intimate contact between the feedstuff and the ruminal environment. This technique has been used to estimate ruminal protein and energy digestibility. Nocek (1988) cited several criteria which should be applied to any in situ procedure.

1. The procedure should be accurate and the results correlated to the actual measured biological response established by in vivo experimentation.
2. The technique should have a high degree of precision within and between laboratories.
3. The technique should require significantly less time and cost compared with in vivo studies.

The in situ technique can meet these criteria but only if certain factors which affect in situ data are standardized among laboratories. The following factors have been shown to affect in situ data: bag porosity, substrate particle size, ratio of substrate weight to bag surface area, extent of microbial contamination, diet of the animal used for incubation of the bags, type of animal, post-ruminal washing procedure, and incubation times.

Bag Porosity

The appropriate porosity of a bag used in an in situ trial should depend upon the particle size of the material placed in the bag. The appropriate porosity should limit the efflux of undegraded feed particles and the influx of ruminal contents. Lindberg and Knutsson (1991) examined the effect of bag porosity on the loss of particulate matter and fiber degradation of forages milled to pass a 1 mm screen. Forages were two grass hays, a clover-grass hay and a straw with a crude protein of 14.7, 14.9, 15.5, and 3.9%, and a NDF concentration of 53.5, 52.7, 49.9, and 80.5%, respectively. Mean bag porosity was 5, 6, 10, 20, or 36 μm. Bag internal dimensions were 10 x 5 cm, and substrate weight was 1 g DM. After soaking the bags in distilled water, water was filtered and residue weighed after drying. There were only small differences in the loss of particulate DM between nylon bags with 5, 6 or 10 μm porosities. The range of DM loss was 3.9 to 9.5% with an average loss of 5.0%. There was a dramatic increase in particulate DM loss when porosity was increased from 10 to 20 μm ($P < .01$). Average particulate DM loss was 10% with 20 μm porosity cloth. Increasing cloth porosity from 20 to 36 μm increased particulate DM loss an additional

11% ($P < .02$). Guinea gress, ground through a 2 mm screen, was incubated in the rumen of a cow in 5 x 12 cm bags having a 37, 53 or 74 µm pore size (Uden and Van Soest, 1984). Neutrel detergent fiber disappeerence increased with increasing pore size (43, 47 and 53%, respectively). In a second experiment by the same authors, timothy hay was incubated in the rumen within 5 x 12 cm bags with pore sizes of 5, 10 or 37 µm. Cell wall disappearance was increased with increasing pore size ($P < .05$) and was 54, 59, and 63%, respectively. Authors concluded that to study cell wall diseppearance in situ, bag pore size should be 37 µm or higher. Nocek (1985) ground soybean meal through a 2 mm screen in e Wiley mill. The ground sample was placed into nylon bags with mean pore sizes of 8, 20, 40, 59, 80 or 102 µm. Bags were soaked for 15 min in 39°C water and DM and nitrogen loss measured. There were no differences ($P > .10$) between pore sizes up to 59 µm for DM and nitrogen disappearance. Bag porosities of 80 and 102 µm resulted in more DM and nitrogen disappearence then other sizes ($P < .05$). The rete of soybean meal DM end nitrogen disappearance was influenced by bag porosity. Bag poresities of 6 and 20 µm gave lower rates of DM and nitrogen disappearance ($P < .05$) when compered with other bag poresities. Estimates of ruminally available protein based on in situ data were compared with in vivo estimates for lactating cows. He concluded that a pore size less than 40 µm underestimated ruminally available protein, but there was no difference between the other porosities.

The effect of bag poresity on in situ disappearance cnefficients appears dependent upon the type of feedstuff being investigated and the nutrient of interest (DM, NDF, or protein). In order to minimize

particulate loss from in situ bags, bag porosity should be matched to the particle size of the substrate. Bag porosity should be between 40 to 60 μm for forages ground through a screen greater than 2 mm (Nocek, 1988). For finely ground grain or forage samples (1 mm), several bag porosities should be evaluated to minimize particulate DM loss while achieving adequate digestion based upon in vivo estimates.

Substrate Weight and Particle Size

It is difficult to interpret the results of experiments attempting to identify the proper substrate weight to bag surface area ratio. The effect of substrate weight is confounded with the effect of substrate particle size and bag porosity. Each of the experiments discussed below used different combinations of bag porosity and substrate particle size. The first experiment could be classified as a small particle size, small porosity experiment. Lindberg (1981) ground hay or barley through a 1 mm screen. Bag porosity was 10 μm and sample size was 5, 10, and 15 mg DM/cm^2. Dry matter and cell wall disappearance tended to be lower in the bags with the largest substrate weight. There was not any difference between the 5 and 10 mg/cm^2 bags for nitrogen disappearance, but nitrogen disappearance was lower ($P < .01$) for the largest sample size when the substrate was barley. Based on these results, Lindberg concluded that a ratio of 10 mg substrate weight/cm^2 was the proper ratio for bags with a pore size of 10 μm, and when sample grind was 1 mm. When two particle sizes were compared (1 vs. 4.5 mm), there was no effect on DM, nitrogen or cell wall disappearance. This was probably due to the small bag porosity, 10 μm, which would limit DM loss from the bags.

A second experiment could be classified as a medium bag porosity, large particle size experiment. Mehrez and Orskov (1977) used three different sizes of bags, 8 x 5 cm, 17 x 9 cm and 25 x 15 cm. The average pore size of the cloth material was 47 μm. Five grams of rolled barley were placed in each bag. The mg of substrate/cm^2 was 54, 16, and 7 for the three bag sizes. Increasing bag size to 17 x 9 cm increased DM disappearance from 38% to 85%. There was no further increase when bag size was 25 x 15 cm. Authors concluded that 17 x 9 cm bags containing 5 g of feed resulting in a ratio of 16 mg of feed/cm^2 was appropriate.

A third experiment could be classified as medium porosity (37 μm) and medium particle size (Guinea grass ground through a 2 mm screen). Uden and Van Soest (1984) measured cell wall disappearance at four substrate weights to bag surface area ratios, 6.5, 13, 25, and 50 mg DM/cm^2. Cell wall disappearance was highest at the smallest ratio and different ($P < .05$) from the other ratios. They concluded that substrate weight to bag surface area ratio should not be more than 6 to 7 mg/cm^2.

Very few of the in situ experiments which quantified the effect of substrate weight to bag surface area ratio compared their results with in vivo data. Erdman et al. (1987) used a ratio of 10 mg/cm^2, a mean bag porosity of 67 μm, and a 2 mm grind. Alfalfa hay, alfalfa haylage, corn silage, and nine energy or protein supplements were incubated in the rumen of lactating cows. Ruminal degredation of nitrogen was measured and compared with in vivo values for protein degradation. The in situ technique underestimated ruminal protein degradation for alfalfa

hay, alfalfa haylage, barley, corn gluten feed, and soybean meal, overestimated ruminal protein degradation for distillers dried grains and brewers dried grains, and accurately predicted ruminal protein degradation for corn and cottonseed meal. The predicted ruminal degradation of alfalfa hay and haylage protein was 9 to 17 percentage units lower than in vivo estimates. The relationship between in situ predicted protein degradation and in vivo estimates was: in vivo value = 1.172 (in situ value) - 9.73, (R^2 = .57, P < .05). They concluded that the in situ procedures used by them underestimated true degradability of protein in feedstuffs of high degradability.

Nacak (1985) compared in situ predicted values of ruminally available protein (RAP) for soybean meal with in vivo values for cattle. The in vivo estimates of RAP from soybean meal were 63 to 79% across all cattle. The in vivo RAP value for soybean meal with lactating cows was 71%. Sample grind (1, 2, or 5 mm) did not effect in situ estimated RAP from soybean meal. Substrate weight to bag surface area ratio did effect estimated RAP. The substrate weight to bag surface area ratio of 12.6 mg/cm^2 had the best estimate of RAP for lactating cows (70 vs. 71%). Other ratios, 25.3 and 37.9 mg/cm^2, had an estimated RAP of 55% which fell in the range of 63 to 79% for in vivo RAP for soybean meal for cattle.

It appears there is not one substrate weight or ratio of substrate weight to bag surface area which will cover all feedstuffs and particle sizes. It seems the smallest sample to bag surface area ratio should be used to facilitate washing and removal of ruminal fluid. In choosing a

bag size, one should consider the amount of residue needed for planned chemical analysis after incubation in the rumen

Variability Among Animals and Bags

In planning any experiment, one needs to estimate cow variation, magnitude of expected treatment differences and the probability level detecting differences. In a classic experiment, Mehrez and Orskov (1977) estimated the variance among sheep, among days on the same sheep, and among bags on the same day on the same sheep. Variation among sheep was the greatest source of variability for DM and nitrogen disappearance. The second largest source of variation was days within the same sheep for DM disappearance and among bags on the same day in the same sheep for nitrogen disappearance. The smallest source of variation was among bags for DM disappearance. Nocak (1985) measured rate of DM disappearance of cottonseed meal using four lactating cows over 6 wk. The smallest source of variability was between duplicate bags within the same cow. The greatest source of variability was week. The differences among weeks averaged for all cows was as large as 10 h^{-1}. The largest variation among duplicate bags was .03 units. Nocak concluded that differences among cows averaged over the 9 wk were not significant. Among the 9 wk, differences among cows were .07, .14, .05, .04, .06 and .09 units. The mean difference among cows within week was .075 units. Animal variability may have been significant if animal was tested each week. In spite of these studies, there is a lack of information on the variability between duplicate bags, among cows or among days within cow. There is no information for nutrients other than DM or nitrogen. The variance components calculated by Mehrez and Orskov (1977) were for

barley. Information is lacking on variance components for in situ experiments utilizing forages and dairy cows.

Washing Procedure and Bag Insertion

Van Keuren and Heinemann (1962) and Merhez and Orskov (1977) both concluded that method of washing did not affect DM disappearance. In most cases, bags are washed under running tap water until the rinsing water is colorless. Nocek (1988) recommends rinsing bags in tap water until the water is clear. He suggests this should take 90 seconds/bag with moderate manipulation. If one wants to use the in situ technique to measure mineral release, a second wash in distilled water would be needed to remove any mineral contamination from the tap water. The current recommended washing procedures are not extensive enough for removing all ruminal bacteria or ruminal fluid. Mason (1969) demonstrated that sonication was more effective for removing microbial contamination of sheep feces than boiling in NDF or ADF solution. Sonication of in situ residues also may be effective in removing microbes. The percent error associated with the determination of residual nitrogen of in situ residues not corrected for microbial contamination can be high. In forages this error was 18 to 44% after 24 h of ruminal incubation (Nocek 1988). Because ruminal microbes contain large amounts of other minerals in addition to nitrogen, in situ mineral release estimates apparent release.

Ruminal insertion and removal of bags is routinely done in two ways. All the bags are placed into the rumen simultaneously and removed sequentially over time, or bags are placed into the rumen sequentially and removed as a group. Introducing bags into the rumen sequentially

and removing them as a group resulted in faster rates of DM and nitrogen disappearance for soybean meal ($P < .05$) than retrieving bags sequentially (Nocek, 1985). Retrieving bags as a group would allow for the mechanization of the washing process. It would minimize disruption of fermentation inside the bag and the rumen. It should reduce variability among days or weeks and bags because washing procedures could be uniform, and contact with the ruminal environment is uninterrupted.

In summary, the in situ technique has the potential to reduce time and cost compared with in vivo studies. Presently, the technique has less precision and accuracy than desirable for predicting in vivo estimates. If a standard methodology would be accepted by the different laboratories using the technique, this could change. The guidelines recommended by Nocak (1986) are a reasonable place to begin. There is a lack of information about the precision and accuracy of the in situ technique for measuring mineral release. If the current situation remains unchanged, that is every laboratory using a different cloth material, different porosity and particle size, then it will be difficult to compare data from different groups. Not enough information exists about the relationship between in situ values and in vivo values collected on the same animals under the same feeding system. Considering all of this, one should exercise caution in interpreting in situ data and applying it to an in vivo situation, unless the data support in vivo values.

In Situ Mobile Nylon Bag Technique

The mobile nylon bag technique was developed by Sauer et al (1983) for use with pigs. Since then it has been used by Kirkpatrick and

Kennelly (1984) and de Boer et al (1988) with dairy cattle. The distinguishing characteristic of this technique is the small bag and substrate weight. Bags were made from nytex with a mean pore size of 48 μm. Bag size was 3.5 x 5.5 cm with a substrate weight of 1 to 2 g. Bags were heat sealed 3 mm from each edge. Internal size was 2.9 x 4.9 cm. The substrate weight to bag surface area ratio was 35 or 70 mg/cm^2 for 1 or 2 g samples.

De Boer et al. (1988) compared the smaller mobile bags (3.5 x 5.6 cm) containing 1 g substrates to large bags (7 x 11 cm) containing 5 g substrates. Both bag types were incubated for 24 h in the rumen of two Holstein cows in midlactation fed a 50:50 concentrate to barley silage diet. Feeds placed within the bags were soybean meal, canola meal, corn gluten meal, fish meal, barley silage, or alfalfa hay. Substrate weight to bag surface area ratio for the large bags was 32 mg/cm^2 and for the small bags was 35 mg/cm^2. There were no differences between the small or large bags for DM or crude protein disappearance during ruminal incubation.

Brittanie et al. (1988) compared 7 x 6 cm bags with bags 5.5 x 1.8 cm. Wheat straw, corn meal, alfalfa meal, soybean meal, or sugarbeet pulp were incubated in the different bag sizes for 0, 3, 18 and 72 h in the rumen of two cows. Dry matter disappearance was similar for bag sizes at 0, 3 and 72 h. The larger bags gave higher values for DM disappearance at 18 h for all feedstuffs. In a second experiment, Brittanie et al. (1988) compared the same bag sizes as the previous experiment at 5 substrate weight to bag surface area ratios (5, 10, 20, 40 and 80 mg/cm^2). Feedstuffs incubated were barley meal, soybean meal,

alfalfa hay, or corn silage. When compared at the same substrate weight, bag size affected disappearance coefficients ($P < .01$) for all the feedstuffs which contradicts the work of de Boer. If bag sizes were compared at the same substrate weight to bag surface area ratios, then results were similar. They concluded that bag size, small versus large, did not affect the rapidly soluble fraction or the undegraded fraction, but the disappearance rate of the slowly disappearable fraction. This conclusion may be premature as it is based only on one time point (h 18).

Kirkpatrick and Kennelly (1984) used the mobile nylon bag technique to estimate ruminal degradable protein and intestinal digestion of undegraded dietary protein. They used the following diets: chopped brome/alfalfa hay and barley (control), control plus canola meal or soybean meal, or meat and bone meal. The nylon bags measured 2.5 x 4 cm with a mean pore size of 40 µm. Two grams of each protein supplement were placed in the bags. The substrate weight to surface area ratio was 100 mg/cm^2. Samples were incubated in the rumen for 15 h, placed in pepsin-HCl for 3 h, and inserted into the duodenum. A comparison between the mobile nylon bag technique and total fecal collection method for the determination of DM and crude protein disappearance gave conflicting results. Crude protein disappearance was estimated accurately by the mobile nylon bag technique ($r = .99$), though slightly lower (-1.9 units). In vivo DM disappearance of the protein supplements was 66%. The DM disappearance estimated by the mobile nylon bag technique was 53%. The depression in DM digestibility was 12 to 14 percentage units depending on the protein supplement. They concluded that the

mobile nylon bag technique could be used to estimate intestinal digestion of crude protein

De Boer et al. (1987) used the mobile nylon bag technique for estimating the intestinal disappearance of ruminal undegradable protein Feedstuffs evaluated were soybean meal, canola meal, corn gluten meal, fish meal, meat and bone meal, and alfalfa hay Feedstuffs were ground through a 1 mm screen Two Holstein cows in mid-lactation fed a 50 50 concentrate barley silage diet and fitted with ruminal and duodenal cannulae incubated the bags in their digestive tract Bags were 3.5 x 5 cm and filled with 1 g of feed, resulting in a 29 mg/cm^2 substrate weight to bag surface area ratio Ruminal incubation was 0, 2, 4, 8, 12 and 24 h. Bags were inserted into the duodenum at a rate of one bag per 45 min. Ruminal incubation in mobile bags was able to differentiate the protein sources into those with high and low degradability Total tract crude protein disappearances were higher than in vivo values, leading them to conclude that true rather than apparent crude protein disappearance was measured De Boer et al (1987) compared two complete diets using this technique Dry matter disappearance at 24 h of ruminal incubation was 69 7 and 72 4% This corresponded well to in vivo values obtained on these diets with conventional digestion methods (71 4 and 71.9%). In contrast, total tract true crude protein disappearance coefficients of the diets estimated in situ were 88 8 and 89 3%, while apparent disappearance coefficients estimated in vivo were 67 2 and 75 9% These authors concluded that their mobile nylon bag technique was a rapid indicator of availability of undegraded dietary protein in

feedstuffs. It appears the diet of the animals used in mobile nylon bag experiments will influence DM and starch disappearance from the bags.

Anzola et al. (1987) fed four cannulated heifers diets containing 50 or 80% concentrate. Alfalfa meal or cottonseed meal was placed into dacron bags (2.5 x 3.5 cm) with a pore size of 50 to 70 μm. The effect of diet on protein and DM disappearance after ruminal incubation (15 h) and postruminal incubation (pepsin-HCl for 3 h followed by passage through the intestines and recovery in the feces) was determined. In the rumen, DM disappearance from mobile dacron bags was greater by heifers receiving the 50% concentrate diet than the 80% concentrate diet ($P < .05$). In the intestines, DM disappearance was less ($P < .05$) while protein disappearance was greater ($P < .05$) by heifers fed the 50% concentrate diet than the 80% concentrate diet. These results indicate that diet can alter both ruminal and post-ruminal disappearance from mobile dacron bags. On these diets, disappearance of DM and starch from mobile dacron bag was 5 to 15% below in vivo disappearance coefficients.

A second experiment (Anzola et al., 1988) examined the influence of substrate particle size on DM, crude protein and starch disappearance from corn in mobile dacron bags. Small mobile bags were incubated in the digestive tract of dairy calves fed 80% concentrate diets. Corn was sieved to obtain particle sizes of <125, 125 to 250, 250 to 500, 500 to 1000 and 1,000 to 2,000 μm. Disappearance was measured after 15 h of ruminal incubation followed by 3 h pepsin-HCl incubation, and after insertion in the duodenal cannula and recovery from the feces. Total tract, ruminal, and small intestinal disappearance of DM, starch and crude protein were greater ($P < .05$) for the smaller particle sizes.

Intestinal disappearance of DM and protein from particles > 500 µm was less than half that of smaller (< 250 µm) particles. Total tract DM disappearance was positively correlated (r = .90, P < .05) with intestinal residence time. For corn grain, particle size limited extent of disappearance of starch and protein.

It seems many of the same factors which influence in situ experiments using large bags also will affect small mobile dacron or nylon bags. Particle size, substrate weight to bag surface area ratio, animal diet, and retention time in the intestines affect disappearance from mobile bags. The mobile nylon bag technique can underestimate ruminal and total tract DM disappearance compared with in vivo values (Kirkpatrick and Kennelly, 1984; Anzola et al., 1987). Yet when bag washing is extensive, in vivo values are similar to in situ values (de Boer et al., 1987). There still is not enough information on the affect of substrate weight to surface area ratio on disappearance of nutrients from mobile bags. There is no information on the use of the mobile nylon bag technique to predict forage DM, cell wall, or mineral release. Since DM and starch disappearance can be depressed in 5.5 x 3.5 cm bags containing grains, bag size should be increased. It is not known what the proper bag size or substrate weight should be to evaluate forages. Based on previous studies with grains, a depression of DM and cell wall disappearance is likely. Future experiments should attempt to evaluate forages with this technique.

CHAPTER III
RUMINAL RELEASE OF MINERALS FROM FORAGES

Introduction

The utilization of minerals from mineral supplements has been reviewed extensively (Ammerman and Miller, 1972; Thompson, 1978), yet studies on the utilization of minerals from forages have been few (Joyce and Rattray, 1979; Reid et al., 1967; Perdomo et al., 1977; Blaney et al., 1982). In the study of mineral metabolism, the total quantity of a mineral in a feedstuff has little significance unless it is qualified by a factor estimating the availability of the mineral to the animal (Seeler, 1972). Animal utilization of a mineral is dependent upon at least two processes: solubilization or release of the element from the feedstuff and absorption of the released mineral (Field, 1981). The extent of release of minerals from forages is a neglected subject. Much of the information on mineral release from forages was collected by laboratories outside the U.S. (Playne et al., 1976, Rooke et al., 1983, Whitehead et al., 1985). The rate and extent to which minerals of forages are released in the rumen apparently depends on the specific mineral, forage species, and location of the mineral in the plant structure (Playne et al., 1978, Rooke et al., 1983, Shockey and Reid, 1984). There is evidence to suggest that differences in mineral availability among plant species may be related to the cell wall content of the species. After 48 h of ruminal fermentation, only 20 to 50% of the

nitrogen, calcium, and phosphorus was released from speargrass and Stylosanthes humilus, both with NDF contents greater than 70% (Playne et al., 1979). In contrast, 65 to 95% of these same minerals were released from alfalfa haylage or ryegrass silage (Rooke et al., 1993). The plant cell wall contains anionic carboxyl groups which can bind metal cations (Molloy and Richards, 1971), thus potentially reducing the release and utilization of minerals from forages.

The objectives of this experiment were:

1. to evaluate the suitability of the in vitro and in situ techniques for measuring the release of minerals from forages,
2. to measure ruminal solubility and rate and extent of release of calcium, magnesium, phosphorus, potassium, copper and zinc from six forage species, and
3. to determine if cell wall concentration influences the rate and extent of mineral released from forages.

Materials and Methods

Experiment 1

The suitability of the first stage of a two-stage in vitro technique (Tilley and Terry, 1963) was compared to that of the in situ technique (Mehrez and Orskov, 1977) for measuring the release of minerals from forages. Alfalfa haylage, dried at 55°C for 48 h and ground through a 2 mm screen, was the substrate for both techniques. Inoculum for the in vitro study was taken 2 to 3 h after morning feeding from a steer receiving bermudagrass hay and a protein supplement. To carry out the in vitro experiment, 500 mg samples were weighed into

100 ml polyethylene centrifuge tubes. Fifty ml of inoculant (40 ml McDougall's buffer + 10 ml ruminal fluid) were added to each tube (Meyer et al., 1972). Tubes were incubated at 39°C for 0, 6, 12, 24, 48, 72 and 96 h, using four replicate tubes per incubation time. After incubation, samples were filtered through No. 54 Whatman filter paper and washed five times with distilled water. Filtered samples were dried at 55°C for 48 h before ashing at 550°C for 4 h. Ash was solubilized with 10 ml of 3 N HCl and boiled gently for 10 min under a watch glass (Association of Official Analytical Chemists, 1980). Solutions were transferred into 25 ml volumetric flasks and brought to volume with distilled water. Calcium, magnesium, and zinc were measured by atomic absorption spectrophotometry (Model 5000, Perkin Elmer, Inc., Norwalk, CT). Phosphorus was determined colorimetrically using an acid molybdate method (Harris and Popat, 1954). Mineral content of residues was corrected with mineral content of blank tubes which contained only inoculum.

To carry out the in situ method, 5.25 g (DM) of dried, ground alfalfa haylage were placed into dacron bags measuring 17 x 9 cm with a mean pore size of 53 µm (Mervelaire White, Erlenger, Blumgart & Co., Inc., New York, NY). Bags were attached to a weighted chain and placed at feeding in the rumen of a steer receiving bermudagrass hay plus a protein supplement. Incubation times were 0, 6, 12, 24, 36, 48 and 72 h with two bags per incubation time. Zero hour bags were not incubated in the rumen but were washed in the same manner as incubated bags. Bags were placed in the rumen over time with all bags removed simultaneously. Upon removal from the rumen, bags were immersed in ice water for 15 min,

then hand-washed until rinse from bags was clear. Bags were dried at 55°C for 48 h. Alfalfa residues at each hour were prepared for mineral analysis as in the in vitro technique. Percent mineral release and DM disappearance were plotted against incubation time for both techniques. The linear regression equations for both techniques as graphed in Figures 1 through 5 were compared using a test for parallelism (Kleinbaum and Kupper, 1978).

Experiment 2

Six forage species of similar maturity, 32 to 37 days regrowth, were hand-harvested near Gainesville, FL in September. Forages selected were 'Florida 77' alfalfa (Medicago sativa, A), 'Florigraze' rhizoma peanut (Arachis glabrata, P), 'Mott' dwarf elephantgrass (Pennisetum purpureum, E), 'Tifton 78' bermudagrass (Cynodon dactylon; 9E), 'Pensacola' bahiagrass (Paspalum notatum, BA) and 'Floreita' limpograss (Hemarthria altissima, L). Forages, except for E, were cut with stainless steel scissors (Ammerman et al., 1982) to a stubble height of approximately 10 cm. Due to the upright and bunch-type morphology of E, leaves were clipped from plants at random, leaving the base and stem intact. All grasses were in the vegetative stage of growth. Alfalfa was at 50% bloom when harvested. Cut forage was placed into cloth bags and dried for 72 h at 60°C. The dried forage was ground to pass a 2 mm Wiley mill screen. Dried, ground forage (5.25 g DM) was placed into 17 x 9 cm dacron bags, having an average pore size of 53 µm (Marvelaire White, Erlanger, Slumgart & Co., Inc., New York, NY). Internal dimensions of the bags were 15.5 x 8 cm, resulting in a substrate weight to bag surface area ratio of 21 mg/cm^2.

Bags were suspended in the rumen of 3 non-lactating Holstein cows receiving long alfalfa hay ad libitum. Bags were attached to a .75 m steel chain and placed in the rumen according to length of planned incubation so that all bags were removed simultaneously. Incubation times were 0 (washed only), 2, 4, 6, 12, 18, 24, 30, 36, and 72 h. There were duplicate bags for each incubation period. Upon removal from the rumen, bags were immersed in ice water for 15 min to stop fermentation immediately. All bags were placed in a cloth sack and washed without soap in a commercial washing machine. After batch washing each bag was hand-agitated in distilled water until effluent from the bag was clear. Bags were dried at 55°C for 48 h and weighed hot.

One gram DM from each bag was ashed at 500°C for 2 h as shown to be optimum ashing time by da Cruz (1983). Ash was solubilized with 15 ml of 3 N HCl and boiled gently under watch glass for 10 min. After boiling, solutions were transferred to 25 ml volumetric flasks and brought to volume with distilled water. Subsequent dilutions for calcium, magnesium and potassium were made by the addition of 1% $LaCl_2$ in 0.1 N HCl. Only 0.1 N HCl was used to dilute samples for phosphorus analysis. Instrumentation for mineral analysis was as in Experiment 1. Neutral detergent fiber was measured without decalin or sodium sulfite (Golding et al., 1995).

The model for the kinetics of fiber disappearance and mineral release was as given by Mertens and Lofton (1980).

$$R = D_o e^{-k(t-L)} + U$$

when $t > L$ and $R = D_o + U$ when $0 < t < L$; where.

R = g of NDF or mineral residue at time after inoculation t,

D_0 = g of slowly released NDF or mineral residue at time t < L,

D_0 = R − U, at t = 0 (residue after washing),

U = g of unreleased NDF or mineral residue at 72 h of incubation,

L = discrete lag time in h and,

k = release rate constant.

The rumen in situ technique allows the mineral content of forages to be separated into three fractions: immediately released, slowly released and not released. The immediately released fraction was released from the forage without undergoing fermentation in the rumen. It was calculated as the initial quantity in the sample minus the quantity remaining in the plant residue at 0 h. The 0 h sample was washed without ruminal incubation. The slowly released fraction was that quantity released during the digestion process in the rumen. It was calculated as the initial quantity in the sample minus the immediately released quantity minus the unreleased quantity. The unreleased fraction was that quantity remaining in the residue after maximum extent of release was reached.

Rates for fiber disappearance and mineral release were calculated using nonlinear iterative procedure of Marquardt in SAS (SAS Users Guide, 1985). Statistical analysis was by least squares analysis of variance using the GLM procedure of SAS, with forage (df = 5), cow (df = 2) and residual (df = 10) as sources of variation in the model. Differences between forages regarding in situ digestion kinetics and

extent of disappearance were assessed by F-protected least significant difference analysis.

Results and Discussion

Experiment 1

Extent and pattern of DM disappearance of alfalfa in the rumen was similar for the in vitro and in situ techniques (Figure 1). However, extent and pattern of mineral release appeared different between the two procedures. Calcium (Figure 2) and magnesium (Figure 3) release in vitro appeared faster than release in situ. Maximum extent of in vitro release for calcium and magnesium was reached by h 6, but 36 h was required for maximum release in situ. The release pattern of phosphorus and zinc are in Figures 4 and 5, respectively. Phosphorus contamination of the in vitro residues appeared to be a problem, as indicated by the erratic pattern of release over time (92 to 72%). Much less phosphorus contamination was evident in the in situ residues, as phosphorus release generally was greater than 90% at all hours. Zinc release in vitro was masked largely by zinc contamination of the residue. Zinc content of the in vitro residue increased rapidly after h 6 such that release was 20, 15, and -19 percent at h 12, 24 and 48, respectively. Zinc contamination of the in situ residues appeared small. Zinc release increased up to h 48.

A test for parallelism indicated that the curves for the in vitro technique and in situ technique were not parallel for DM, calcium, magnesium and zinc release ($P < .05$). This suggests that a separate equation was needed for each technique. Curves for phosphorus release

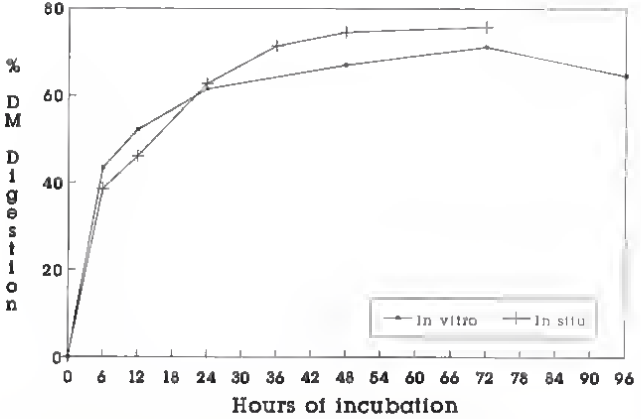

Figure 1. Digestion of DM from alfalfa incubated using an in vitro or an in situ technique.

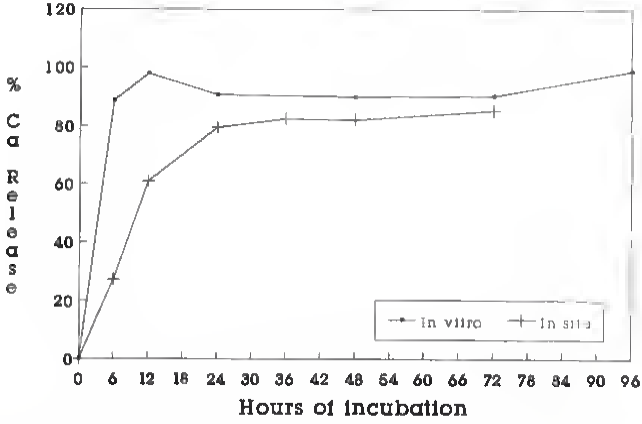

Figure 2- Release of calcium from alfalfa incubated using an in vitro or an in situ technique.

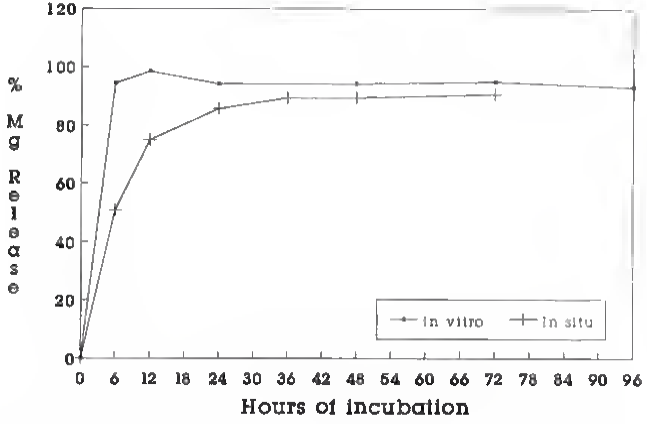

Figure 3. Release of magnesium from alfalfa incubated using an in vitro or an in situ technique

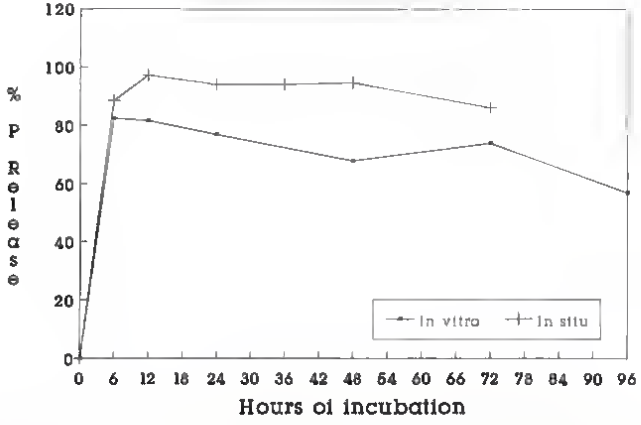

Figure 4. Release of phosphorus from alfalfa incubated using an in vitro or an in situ technique.

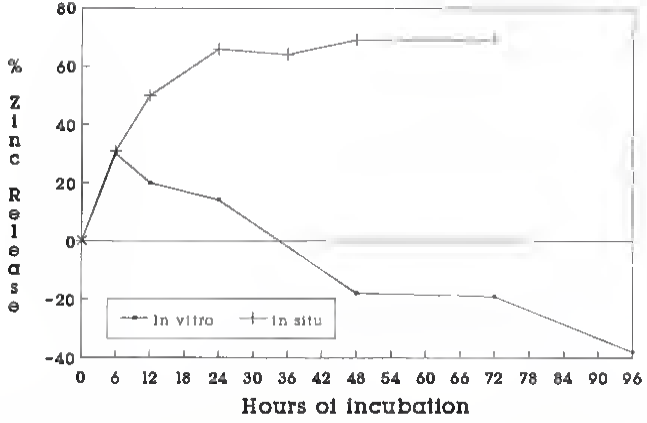

Figure 5. Release of zinc from alfalfa incubated using an in vitro or an in situ technique.

were parallel. These results indicate that the first stage of a two-stage in vitro technique (Tilley and Terry, 1963) was not as suitable for the evaluation of phosphorus and zinc release from forages as the in situ technique. Residues likely became contaminated with minerals from ruminal fluid, McDougall's buffer and microorganisms. The in situ technique likely is not without contamination, however, contamination appeared to be small relative to the amount of mineral remaining in the residue. This was suggested by the high extent of release for all minerals, greater than 80% for calcium, magnesium, and phosphorus, and greater than 68% for zinc. The in situ technique yielded more consistent results for zinc and was selected to evaluate mineral release from six forage species.

Experiment 2

The initial CP, NDF, and mineral concentrations of the six forages varied with forage species as expected (Table 3-1). Quality of the grasses appeared high based on their low NDF and high CP concentrations. Mineral concentrations were generally as expected except that K was higher in potassium (5.8 vs. 3.6%) and magnesium (.48 vs .18%) than seen previously at this station (Montalvo et al., 1987). Differences among the same species grown in different years can be large. The 'Pensacola' bahiagrass harvested in 1986 for this study was higher in calcium, magnesium and copper but lower in phosphorus and potassium than previously reported by Rojas-Osachas (1985). Plant species growing on similar soil type and under similar environmental conditions are different in mineral content. Under temperate conditions, legumes were higher in calcium and magnesium than were grasses (Todd, 1961, Kincad

TABLE 3-1. CHEMICAL COMPOSITION OF SIX FORAGE SPECIES

Forage	CP	NDF	Ca	Mg	K	P	Cu	Zn
	% OM						ppm	
Alfalfa	20.5	47.6	1.46	.39	2.90	.22	11	22
Rhizoma peanut	16.2	50.3	1.31	1.05	2.59	.24	7	25
Dwarf elephantgrass	15.6	59.3	.37	.48	5.83	.20	16	28
Bermudagrass	9.2	68.0	.51	.62	2.09	.16	7	22
Bahiagrass	14.0	67.2	.43	.47	1.65	.17	9	24
Limpograss	12.0	69.3	.47	.43	1.85	.23	11	37

and Cronrath, 1983). In this study only calcium was consistently higher in legumes than grasses. A similar pattern was observed by Reid et al. (1987) with alfalfa, red clover, orchardgrass and ryegrass. Species and varietal differences in mineral content of forages also have been observed under subtropical (Rojas-Osechas, 1985) and tropical conditions (Gomide, 1978; Reid and Horvath, 1980).

Percent immediately released mineral varied with the forage species and the mineral (Table 3-2). The range among the six forages was -20 to 53% for calcium, 72 to 92% for magnesium, 98 to 100% for potassium, 46 to 84% for phosphorus, 51 to 89% for copper and 7 to 43% for zinc. The ranking of the minerals for immediate release in the rumen averaged across forages was potassium (100%) > magnesium (82%) > copper (71%) > phosphorus (56%) > calcium (29%) > zinc (26%). This agrees in part with Rooke et al. (1983) who ranked rapid mineral release in alfalfa haylage and ryegrass haylage as sodium > magnesium > potassium > copper > calcium > zinc > phosphorus. Playne et al. (1978) ranked mineral release from hays after 12 h of ruminal incubation as: potassium > magnesium > sodium > phosphorus > calcium. From these studies plus our study, it can be concluded that a greater percentage of potassium, magnesium and sodium will be rapidly released from forages while a smaller percentage of calcium and zinc is immediately soluble. Percentage of phosphorus and copper immediately released could be high or low depending on the forage species.

The in situ technique estimated the amount of rapidly released mineral in forages as accurately as unchemical separation. Whitehead et al. (1985) unchemically separated forages into cell wall and non-cell

TABLE 3-2. LEAST SQUARES MEANS FOR IMMEDIATELY RELEASED MINERAL OF 51X FORAGE SPECIES WASHED IN WATER (INCUBATION TIME = 0 H)

Forage	Ca	SEM	Mg	SEM	K	SEM
			%			
Alfalfa	46.7[a]	1.23	71.8[a]	1.39	99.9[a]	.04
Rhizoma peanut	15.7[b]	1.23	79.0[b]	1.39	88.4[b]	.04
Dwarf elephantgrass	20.0[c]	1.23	88.7[c]	1.39	99.9[a]	.04
Bermudagrass	52.9[d]	1.23	92.1[d]	1.39	99.9[a]	.04
Bahiagrass	25.3[e]	1.23	75.6[eb]	1.39	99.9[a]	.04
Limpograss	52.2[d]	1.23	85.7[c]	1.39	99.9[a]	.04

Forage	P	SEM	Cu	SEM	Zn	SEM
			%			
Alfalfa	75.9[a]	2.69	88.9[a]	4.04	25.8[a]	6.14
Rhizoma peanut	46.1[b]	2.69	50.6[b]	4.04	19.1[ab]	6.14
Dwarf elephantgrass	83.7[a]	2.69	84.4[a]	4.04	7.3[b]	6.14
Bermudagrass	50.5[bc]	2.69	69.9[a]	4.04	43.1[c]	6.14
Bahiagrass	55.1[c]	2.69	63.1[c]	4.04	33.8[ac]	6.14
Limpograss	91.9[a]	2.69	70.0[c]	4.04	26.6[a]	6.14

[a,b,c,d] [a]Means in the sumn column with different superscripts are different

(P <.05 for Ca, Mg, K, P, Cu), (P < .06 for Zn).

wall fractions using rotating cylinders. This technique eliminates possible mineral contamination from NDF solution or ruminal microbes. They ranked minerals in the cell wall and non-cell wall fractions of two legumes and two grasses based on their solubility in water. The ranking was potassium > phosphorus > magnesium > copper > calcium > zinc when averaged across forage species. It seems that phosphorus release was the most difficult to estimate using the in situ technique. The release of phosphorus has been low (Playne et al., 1978, Reeke et al., 1983) or high (Whitehead et al., 1985) or intermediate as in our study. These differences were likely the result of the high phosphorus content of ruminal microorganisms and ruminal fluid (Durand and Kawashima, 1980), which could contaminate in situ residues.

Dwarf elephantgrass appeared to have a strong affinity for binding calcium. Calcium concentration increased 20% from the initial sample (Table 3-2). Possible binding agents include pectin and lignin (Molloy and Richards, 1971). These structural carbohydrates may bind zinc also, as the release of elephantgrass zinc was considerably lower than the other forages.

Extent (72 h) of DM and NDF disappearance and mineral release in the rumen is in Table 3-3. The two legumes, A and P, plus E contained the most digestible DM and NDF. The DM and NDF disappearence coefficients for E were higher than the other forages, even the legumes, A and P. The fact that about 80% of the plant was leaf may help account for these high coefficients. Release of the six minerals ranged from 59 to 78% for calcium, 88 to 99% for magnesium, > 99 for potassium, 64 to 95% for phosphorus, 70 to 94% for copper and 53 to 81% for zinc. These

TABLE 3-3. LEAST SQUARES MEANS FOR EXTENT OF DM AND NDF DISAPPEARANCE AND MINERAL RELEASE OF SIX FORAGE SPECIES INCUBATED IN THE RUMEN (INCUBATION TIME = 72 H)

Forage	DM	SEM	NDF	SEM	Ca	SEM	Mg	SEM
	%							
Alfalfa	77.8[c]	1.13	51.9[a]	2.17	59.3[a]	3.01	95.2[a]	.31
Rhizoma peanut	73.5[b]	1.13	58.7[a]	2.17	66.0[ab]	3.01	99.2[b]	.31
Dwarf elephantgrass	84.2[c]	1.13	78.1[b]	2.17	73.7[bc]	3.01	97.9[c]	.31
Bermudagrass	95.2[d]	1.13	56.9[ac]	2.17	78.1[c]	3.01	99.5[d]	.31
Bahiagrass	56.2[a]	1.13	51.7[cd]	2.17	94.5[ab]	3.01	88.2[e]	.31
Limpograss	90.3[a]	1.13	49.5[d]	2.17	77.2[c]	3.01	93.1[f]	.31

Forage	K	SEM	P	SEM	Cu	SEM	Zn	SEM
	%							
Alfalfa	99.9	.01	85.1[c]	1.40	92.3[a]	1.33	79.4[a]	1.85
Rhizoma peanut	99.9	.01	94.8[b]	1.40	89.9[a]	1.33	80.5[a]	1.85
Dwarf elephantgrass	99.9	.01	91.6[b]	1.40	94.3[a]	1.33	75.5[a]	1.85
Bermudagrass	99.9	.01	65.3[c]	1.40	75.8[b]	1.33	92.1[b]	1.85
Bahiagrass	99.9	.01	94.3[c]	1.40	81.7[c]	1.33	53.0[c]	1.85
Limpograss	99.9	.01	80.7[d]	1.40	99.5[d]	1.33	97.2[b]	1.85

[a,b,c,d,e,f]Means in the same column with different superscripts are different ($P < .05$)

values are higher than those reported by Playne et al (1978) for two tropical legumes and two tropical grasses. This may be a result of differences in washing procedure between laboratories, hand washing only (Playne et al., 1978) vs. mechanical washing followed by distilled water washing. In a study using similar washing procedures to ours (Rooke et al., 1983), the release of minerals from silages agreed with our study, 77 to 99% for calcium, 88 to 94% for magnesium, 90 to 91% for potassium, 66 to 84% for phosphorus, 79 to 90% for copper, and 74 to 88% for zinc. With the exceptions of calcium and zinc, mineral release coefficients were greater than those for DM and NDF disappearance. Therefore, a majority of magnesium, potassium, phosphorus, and copper likely reside in the cell contents or a highly digestible portion of the cell wall such as the mesophyll and phloem (Akin and Burdick, 1975). Using a scanning electron microscope, McManus et al. (1977) found only 19 to 38% of the ash content of tropical grasses to be associated with the plant cell wall. Forages having the highest DM disappearance coefficients, namely A, P, and E, had higher release coefficients for phosphorus, copper, and zinc. Release coefficients for calcium, magnesium, and potassium were similar between the higher quality and lower quality forages. It appears that minerals from high quality forages will be released extensively for possible animal metabolism. Forages of average DM disappearance such as BA and L (58 to 60%) can release most of there minerals. Extent of calcium release was lowest for A. The effect of stage of growth on mineral release needs to be examined before we conclude that alfalfa calcium was less available than calcium in other forage species. Alfalfa was at a different physiological maturity than

the other forages (flowering vs. vegetative). Potassium release essentially was complete (99.9%) for all forage species.

Maximum release of calcium and magnesium often required longer incubation times than that required for potassium, phosphorus, copper or zinc (Table 3-4). Playne et al. (1979) also found a longer incubation time was required for maximum calcium release (48 h). The longer times for maximum release of calcium could be explained by its function in plants. Calcium is important for the stability of cell walls and membranes. It is the major cation found in the middle lamella (Mackie and Therion, 1984). Therefore, as the plant cell wall is digested, increasing quantities of calcium likely are released.

Percentage of minerals slowly released in the rumen for the six forages is in Table 3-5. It is the slowly released mineral which possibly could be affected when ruminal fermentation is manipulated. The range of slowly released mineral for these forages was 13 to 74% for calcium, 4 to 23% for magnesium, 0 to 49% for phosphorus, 0 to 39% for copper and 19 to 69% for zinc. Similar values have been reported for the portion of mineral associated with the plant cell wall (Whitehead et al., 1995). Plant cell wall mineral for alfalfa, white clover, tall fescue and perennial ryegrass had ranges of 11.3 to 51.8% for calcium, 5.4 to 27.6% for magnesium, 0.1 to 1.7% for potassium, 3.0 to 6.9% for phosphorus, 18 to 44% for copper, and 24 to 78% for zinc. Among the forages, P had the greatest mean percent (37%) of slowly released minerals. Of the four grasses and A, only small amounts of magnesium ($\leq$ 23%), phosphorus ($\leq$ 15%), and copper ($\leq$ 19%) were released slowly.

TABLE 3-4. INCUBATION TIME REQUIRED TO REACH MAXIMUM EXTENT OF DM AND NDF DISAPPEARANCE AND MINERAL RELEASE FOR SIX FORAGE SPECIES

Forage	DM	NDF	Ca	Mg	K	P	Cu	Zn
	hour[A]							
Alfalfa	36-72	36-72	12-18	12-30	2	4-6	4-12	12-18
Rhizoma peanut	24-36	24-72	18-24	18	2	12-18	12-30	12
Dwarf elephantgrass	30-72	36-72	36-72	24-36	2	24	30-36	30-36
Bermudagrass	72	72	18-24	18-24	2	2-4	12	6-12
Bahiagrass	72	72	30-36	36	2	2-4	6	6-18
Limpograss	36-72	36-72	30-36	24-30	2	2-6	2-6	12-24

[A]Range in time required to reach maximum extent represent differences among animals

TABLE 3-5. LEAST SQUARES MEANS FOR SLOWLY RELEASED MINERAL OF SIX FORAGE SPECIES INCUBATED IN THE RUMEN (INCUBATION TIME = 72 H)

Forage	Ca	Mg	K	P	Cu	Zn	Average
				%[a]			
Alfalfa	13	23	0	9	4	54	17
Rhizoma peanut	50	20	0	49	39	62	37
Dwarf elephantgrass	74	11	0	8	10	69	29
Bermudagrass	25	4	0	15	6	19	12
Bahiagrass	39	13	0	9	19	19	17
Limpegrass	25	7	0	0	0	41	12
Average	39	13	0	15	13	44	

[a]Calculated by difference (initial quantity - rapidly released quantity - unreleased quantity) ÷ initial quantity.

All potassium was rapidly released. This could indicate that these minerals were present primarily in the cell contents as ionic compounds or part of water soluble plant components. Thirty-eight percent of the released calcium and 44% of the released zinc fell in the slowly released category, possibly indicating cell wall association.

Legumes had faster DM disappearance rates than grasses (Table 3-6). Release rate constants for NDF disappearance were not different among forages, yet there was a trend for legume NDF to have higher rate constants than grass NDF. This trend also has been observed in forages grown under temperate conditions (Varga and Hoover, 1983). Rate of mineral release from legumes was at least twice that from grasses. Rate constants were not different between the two legumes nor different among the four grasses. Rooke et al. (1993) reported no differences between alfalfa and ryegrass haylage in the fractional rates of release of seven minerals. Our six different forage species had a wide range of NDF concentration and digestibilities, which could explain the differences observed. There appears to be two pools of concentration for each of the six minerals except potassium. The larger pool mineral is quickly released, and the smaller pool slowly released. Considering only these grasses, release rate constants for the minerals in the smaller pool were similar to that for NDF. However, release rate constants of minerals were greater than those for NDF or DM of the two legumes.

Due to the high extent of mineral release and large proportion of immediately released mineral in these forage species at their stage of maturity, it was likely that magnesium, potassium, phosphorus, copper

TABLE 3-6 LEAST SQUARES MEANS FOR RELEASE RATE CONSTANTS OF SLOWLY RELEASED DM, NDF AND MINERAL FROM SIX FORAGE SPECIES INCUBATED IN THE RUMEN

Forage	DM	SEM	NDF	SEM	Ca	SEM
	h^{-1}					
Alfalfa	.17[a]	.04	.10	.02	.25[a]	.03
Rhizome peanut	.11[a]	.04	.10	.02	.22[a]	.03
Dwarf elephantgrass	.03[b]	.04	.06	.02	.09[b]	.03
Bermudagrass	.07[b]	.04	.07	.02	.09[b]	.03
Bahiagrass	.07[b]	.04	.07	.02	.08[b]	.03
Limpograss	.08[b]	.04	.07	.02	.08[b]	.03

Forage	Mg	SEM	P	SEM	Cu	SEM	Zn	SEM
	h^{-1}							
Alfalfa	.26[a]	.02	—[c]	—	—	—	.36[a]	.03
Rhizome peanut	.27[a]	.02	.44[a]	.03	.20[a]	.03	.37[a]	.03
Dwarf elephantgrass	.06[b]	.02	.09[b]	.03	.07[b]	.03	.09[b]	.03
Bermudagrass	.10[b]	.02	—	—	.10[b]	.03	—	—
Bahiagrass	.09[b]	.02	—	—	—	—	—	—
Limpograss	.09[b]	.02	—	—	—	—	.13[b]	.03

[a,b]Means in the same column with different superscripts are different ($P < .05$)

[c]Rate constants were not calculated for minerals with extent of release reached in less than 12 h.

and zinc were not associated tightly with the plant cell wall. This does not imply that these minerals were highly available for absorption from the rumen. It does imply that these minerals were available to the ruminal microorganisms. Bacterial action can render minerals unavailable in the rumen. Bacterial cell wall surfaces are negatively charged at neutral pH (Mackie and Therion, 1984). Gram positive bacterial cell walls can readily exchange metal ions with the environment. Gram negative bacterial cell walls contain magnesium and calcium as important components of their outer membrane and they help to maintain outer membrane structure (Mackie and Therion, 1984). Calcium, magnesium, zinc and copper can accumulate in bacterial cell walls. Increasing the quantity of these minerals in solution or the diet has increased their concentration in the bacterial cell wall (Fitt et al., 1974; Fitt and Hatton, 1974; Durand and Kawashima, 1980). Zinc and copper concentration of ruminal microorganisms can be two to four times greater than the dietary concentration (Durand and Kawashima, 1980). It has been suggested that the ruminal microbial population may be responsible for the reduced availability of dietary magnesium observed in ruminants compared with nonruminant species (Fitt et al., 1974).

Minerals contribute to the regulation of physiochemical factors which can affect ruminal fermentation. One such factor is buffering capacity of the ruminal environment. Dietary fiber can buffer the gastrointestinal tract by increasing saliva production and via the cation exchange capacity of the fiber itself. Cation exchange capacities of the plant cell wall differs among forage species and legumes have higher cation exchange capacities than grasses (McGarney et

al., 1983). Alfalfa had greater in vitro acid buffering capacity than grasses (Wohlt et al., 1987) and grass species were not equal in acid buffering capacity (Jasaitis et al., 1987). Acid buffering capacity of forages has been correlated with total ash, r = .88, and total cations, r = .87 (Jasaitis et al., 1987). It was likely that variability among forage species for acid buffering capacity was due to differences in amount of mineral released. This could be calculated as mineral concentration times percent released mineral times DM intake. For example, the amount of magnesium released from alfalfa hay used in this experiment would be 3900 mg/kg DM x .95(release factor) = 3705 mg/kg DM. From this study it appears that forage species was an important factor influencing the amount of mineral released in the rumen. Additional work needs to be done examining the effect of stage of maturity on ruminal mineral release. It is possible that low mineral concentrations in a forage plus low ruminal release could result in a less than optimal supply of certain minerals for maximum microbial fermentation.

It appears that significant amounts of calcium were associated with the plant cell wall based on its lengthy fermentation time and small amount of rapidly released mineral. Zinc could be associated with the plant cell wall but may be part of plant proteins. Though the forages contained a high percentage of slowly released zinc, ranging from 25 to 68 percent across the six forage species, extent of digestion was reached within 24 h except for E. This would be similar for protein digestion, while extensive ruminal cell wall digestion usually requires 35 to 96 h.

The rumen appears to be the major site of mineral release from these forages. The in situ technique was adequate for detecting differences among forage species in the pattern of mineral release. Forages possessing the highest NDF concentration, BE, BA, and L, had lower DM and NDF disappearence coefficients compared to A, P, and E. These forages, BE, BA, and L, also had lower release coefficients for phosphorus, copper and zinc compared to A, P, and E. Neutral detergent fiber concentration did not influence rate and extent of mineral release as greatly as plant family (legumes vs. grasses).

CHAPTER IV
EXTENT AND SITE OF MINERAL RELEASE FROM SIX FORAGE SPECIES INCUBATED IN MOBILE DACRON BAGS

Introduction

Efficient ruminal microbial growth and fermentation requires an adequate supply of minerals, in addition to an optimum mixture of energy and protein. Minerals contribute to the regulation of ruminal osmolality, dilution rate and buffering capacity (Mackie and Therion, 1984). For these reasons it is prudent to develop a technique by which release of feed minerals in the digestive tract can be estimated. Nocek (1988) reviewed the extensive effort devoted to developing techniques to predict ruminal protein and energy digestibility. Yet optimum utilization of these ruminally degradable nutrients will depend upon a proper balance of all dietary nutrients. Mackie and Therion (1984) concluded there was little information on the elution or solubilization of minerals from feed particles in the rumen. It is this released mineral fraction which will supply ruminal microbial requirements.

The mobile nylon bag technique has been used to estimate the digestion of protein feedstuffs in pigs (Sauer et al., 1983) and dairy cattle (Kirkpatrick and Kennelly, 1984; de Boer et al., 1988). Estimation of protein and energy digestion in the stomach and intestines was possible with this technique. The objectives for this experiment were

1. to determine if the mobile dacron bag technique can be used to measure DM disappearance and mineral release from forages,
2. to measure the extent of DM disappearance and mineral release from forage species within the rumen, abomasum and intestines using the mobile bag technique, and
3. to determine if forage by digestion site interactions are present for release of specific minerals.

Materials and Methods

Three non-lactating Holstein cows were fitted with ruminal and duodenal cannulas. The duodenal cannula was T-shaped and made of silastic tubing. Its internal diameter was 2 cm and its length was 13 cm. An incision was made caudal to the last rib on the right side of the animal, exposing a horizontal segment of the duodenum. A 2.5 cm incision was made in this section of the duodenum and the wings of the T-shaped cannula were folded and placed within the lumen of the duodenum. A purse string suture around the barrel of the cannula held it in contact with the duodenum. This location placed the cannula approximately 1 m caudal to the pylorus. The cannula was exteriorized through a second incision in the body wall. A 5 cm sleeve of silastic tubing was placed around the barrel of the cannula beginning at the body wall. The sleeve was held in place with silastic glue and elastic tape. The purpose of the sleeve was to limit movement of the cannula. By limiting cannula movement, it was hoped that the duodenum would remain in close contact with the inner muscle layer of the body wall. This likely would reduce peristaltic movement but would increase the chance of a leak-proof connection between the duodenum and the body wall. The

duodenal cannulas remained in place for 90 d. After their removal, the incision closed within 48 h and the animals were returned to the herd.

Dacron material having an average pore size of 53 μm was used to prepare the mobile bags (Narvelaire White; Erlanger, Blumgart and Co., Inc., New York, NY). Bags were cut out, sewn with nylon thread, and the seams glued with rubber cement. An initial attempt was made to measure forage digestion postruminally by inserting bags into the abomasum and collecting them in the feces. One g of dried, ground bermudagrass was placed into 9 x 3 cm bags. Two bags were inserted into the abomasum of a cow fitted with an abomasal cannula. After 72 h, bags had not exited the digestive tract. Upon dosing 4 L of mineral oil into the rumen over a 2 d period, bags appeared in the feces. This was repeated a second time with smaller bags, 5 x 3 cm, with similar results. Sauer et al. (1983) observed the same problem with pigs and abandoned the idea of abomasal insertion of small nylon bags (5 x 3 cm). Bags were inserted into the duodenum via cannula in future experiments.

To determine the proper bag size and amount of substrate to place in a bag, dacron bags measuring 10 x 3 cm and 9 x 3 cm were filled with 1, 1.5 or 2 g of dried, ground bermudagrass. Bag size was not an important factor, as both size bags were collected in the feces after insertion into the duodenum. Sample weight did affect residence time of the bags. Bags containing 1 to 1.5 g of dried forage passed through the intestines in 11 to 36 h. Bags containing 2 g of forage passed out in 36 to 72 h. This latter passage was slow, as 5 x 3 cm bags containing 2 g of protein feedstuffs had residence time in the intestine of 6 to 32 h (Kirkpatrick and Kennelly, 1984). Forages within the bags tended to

swell when wet. This hydration increased the weight of the total sample. Differences in the amount of hydration of forages versus protein feeds may explain why the same sample size had different rates of passage. It was decided to limit sample size to 1 g DM.

Experiment 1

Because bags would not pass through the pylorus naturally, a method to simulate abomasal conditions was needed. An in vitro and an in situ method were evaluated. Initially, forty dacron bags each containing 1 g of dried, ground bermudagrass were incubated in the rumen of a ruminally fistulated, non-lactating Holstein cow for 24 h. These bags were used to test in vitro and in situ conditions simulating in vivo abomasal conditions.

To carry out the in vitro experiment, twenty bags of the forty bags were placed individually into 100 ml of an HCl-pepsin solution (Tilley and Terry, 1963) with continuous stirring. Four bags were removed at each of the incubation times: 0, 10, 30, 60 and 120 min. Bags were washed in distilled water, dried at 55°C for 48 h, and weighed hot. Forage residues from bags were recovered and ashed at 500°C for 2 h which was determined as optimal for mineral recovery by da Cruz (1983). Ash was solubilized with 5 ml of 3 N HCl and boiled gently for 10 min under watch glass. After boiling, solutions were transferred to 10 ml volumetric flasks and brought to volume with distilled water. Digested residues were analyzed for calcium, magnesium, and zinc by atomic absorption spectrophotometry (Model 500, Perkin-Elmer, Norwalk, CT). Phosphorus was determined colorimetrically (Harris and Popat, 1954).

The remaining 28 bags were used for the in situ study. Bags were inserted two at a time into the abomasum of an abomasally cannulated cow consuming alfalfa hay ad libitum. Incubations were 0, 15, 30, 60, or 120 min with four bags per incubation time. After removal from the abomasum, bags were handled as in the in vitro study. With both methods, in situ or in vitro incubation, the 0 h bags represented ruminal incubation for 24 h only. Analysis of variance was performed using the ANOVA procedure of SAS (SAS Users Guide, 1985). The terms contained in the model were method, time, replication, method by time, method by replication and residual. Least squares means for method and time were compared using F-protected LSD.

Experiment 2

Three non-lactating Holstein cows fitted with ruminal and duodenal cannulas as described previously were fed long alfalfa hay ad libitum. Six forages were hand harvested with stainless steel scissors (Ammerman et al., 1982). Forages harvested were 'Florida 77' alfalfa (Medicago sativa, A), 'Florigraze' rhizmea peanut (Arachis glabrata; P), 'Mott' dwarf elephantgrass (Pennisetum purpureum; E), 'Pensacola' bahiagrass (Paspalum notatum, BA), 'Tifton 78' bermudagrass (Cynodon dactylon, BE) and 'Floralta' limpograss (Hemarthria altissima, L). All forages were clipped at 32 to 37 d regrowth. Alfalfa was at 50% bloom whereas all other forages were in the vegetative stage of growth. Wet forage was dried in cloth bags for 72 h at 55°C. The dried forage was ground to pass a 2 mm screen in a Wiley mill.

Forage (1.08 g DM) was placed into 8 x 3 cm dacron bags. Internal diameter of the sealed bag was 7 x 2.5 cm, resulting in a substrate

weight to bag surface area ratio of 31 mg/cm^2. Twelve bags containing the same forage were placed in a nylon mesh sack and inserted into the rumen to incubate for 24 h. The nylon sack was secured to a weighted chain and placed in the ventral rumen sac. Bags containing the six different forages were assigned randomly to cows for six 5 d periods so that each cow received a different forage during the same period. After 24 h, bags were removed from the rumen and immersed in ice water for 15 min to stop fermentation. Four bags were washed and dried. Eight bags per cow were placed individually into 100 ml acid-pepsin solution and stirred by magnetic stir bar for 1 h. After removal, four bags were washed and dried and the remaining four were individually inserted through the duodenal cannula 30 minutes apart. Bags were collected in the feces and immersed in ice water for 15 min. All bags were washed in a commercial washing machine without soap followed by hand agitation in distilled water. Bags were dried at 55°C for 48 h and weighed hot. Mineral analysis was as in experiment 1.

Statistical analysis was by least squares analysis of variance with the GLM procedure of SAS (SAS Users Guide, 1985). The effect of digestion site for each mineral of each forage species was tested with a model containing cow (df = 2), digestion site (df = 2), cow by digestion site (df = 4) and residual (df = 9) as sources of variation. Differences among forage species were tested with a factorial model containing forage (F), cow (C), digestion site (S), F by C, F by S, C by S and F by C by S as sources of variation. Least squares means were tested for significance by F-protected LSD ($P < .05$).

Results and Discussion

Experiment 1

Regardless of the method, in vitro acid-pepsin or in situ abomasal incubation, release of minerals beyond that occurring in the rumen was small except for phosphorus (Table 4-1). Incubation in acid-pepsin solution increased phosphorus release approximately 9 percentage units while abomasal incubation increased phosphorus release 11 percentage units above ruminal incubation alone. Calcium, magnesium and zinc release increased from 1 to 6 percentage units after acid-pepsin or abmeasal incubation. Zinc release was inconsistant when bags were incubated in the abomasum. Zinc release decreased after abomasal incubation for 30 and 60 minutes indicating that the bag residue increased in zinc concentration. This may have been a problem of incmeplete washing of the bags.

The two methods were evaluated on variability among bags at each incubation time. An average SEM was calculated for each mineral at each incubation time for each method. Abomasally-inserted bags had larger average SEM than bags incubated in acid-pepsin especially for phosphorus and zinc, but lower for calcium. Inconsistant wetting of the bags with abomasal fluid did occur and may explain the increased variability between bags inserted into the abomasum. Acid-pepsin incubation was selected as the method of choice to evaluate six forages due to ease of measurement and reduced variability. Incubation time was set at one hour for experiment 2 because, within the acid-pepsin method, maximum mineral release generally had been reached within 60 min except for zinc. There was a method by time interaction ($P < .05$) for zinc

TABLE 4-1 MINERAL RELEASE FROM BERMUDAGRASS INCURATED IN THE RUMEN FOR 24 H IN MOBILE DACRON BAGS FOLLOWED BY INSERTION INTO THE ABOMASUN OR INCUBATION IN ACID-PEPSIN SOLUTION

Incubation Time	Acid-Pepsin Ca	SEM	Mg	SEM	P	SEM	Zn	SEM
(min)	%							
0	89.3	.81	93.8	.58	64.0	.77	87.0	3.54
15	90.1	.91	94.2	.61	73.3	2.03	85.0	1.54
30	90.0	2.83	94.1	.96	72.7	.63	88.6	1.49
60	92.7	.13	95.4	.16	67.7	.46	86.0	.28
120	93.0	.37	95.6	.02	73.4	1.28	90.5	.31
Mean	91.0[a]	1.01	94.6[a]	.47	70.2[b]	1.03	87.4[a]	1.43

Incubation Time	Abomasum Ca	SEM	Mg	SEM	P	SEM	Zn	SEM
(min)	%							
0	89.3	.81	93.8	.58	64.0	.77	87.3	3.54
15	94.1	.22	94.9	.84	69.5	.79	86.8	.25
30	94.2	.11	94.7	.19	70.0	4.50	71.0	5.27
60	93.9	.14	94.7	.09	70.0	2.88	77.9	7.65
120	94.9	.56	95.6	.44	75.4	3.52	91.2	4.41
Mean	93.3[b]	.37	94.7[a]	.43	69.8[a]	2.49	82.8[a]	4.22

[a,b]Means in same column with different superscripts are different ($P < .05$).

release. Method by time interaction was not significant for the other minerals.

Experiment 2

Chemical composition of the six forages varied with species (Table 4-2). Percent NDF ranged from 49 to 69% DM. Grasses were harvested from fertilized experimental plots which were well managed.

Forage, digestion site and cow affected DM, calcium, magnesium and phosphorus release from dacron bags (Table 4-3). Because data for forage is pooled across digestion site and data for digestion site is pooled across forage, the F by S interaction is of particular interest. Significant interactions will be discussed using Tables 4-4, 4-5, 4-7 and 4-8 in the following pages.

Twenty-four h extent of DM disappearance in the rumen varied from 45% for BA to 71% for A (Table 4-4). Ruminal incubation accounted for 86 to 100% of total tract digestion. The relatively short incubation time reduced DM disappearance of BA and L greater than the other forages compared to DM disappearance measured with 17 x 9 cm bags incubated in cows on the same diet (Chapter III). Forages with higher NDF concentrations such as BE, BA, and L would require more time in the rumen for complete digestion since fiber is the slowest digesting nutrient. Dry matter disappearance was increased by acid-pepsin incubation only for A. This forage was markedly higher in crude protein than the four grasses (Table 4-3). The high protein concentration coupled with the short ruminal incubation time could allow some protein to escape ruminal degradation which would be hydrolyzed in acid-pepsin solution and contribute to the observed DM loss. The lack of effect by acid-pepsin

TABLE 4-2. CHEMICAL COMPOSITION OF SIX FORAGE SPECIES

Forage	CP	NDF	Ca	Mg	K	P
	% DM					
Alfalfa	20.5	47.9	1.46	.39	2.90	.22
Rhizoma peanut	16.2	50.1	1.31	1.05	2.59	.24
Dwarf elephantgrass	15.6	59.3	.37	.48	5.83	.20
Bermudagrass	9.2	68.0	.51	.62	2.09	.16
Bahiagrass	14.0	67.2	.43	.47	1.66	.17
Limpograss	12.0	69.3	.47	.43	1.85	.23

TABLE 6-3. SIGNIFICANCE LEVELS FOR MAIN EFFECTS, AND INTERACTIONS FOR DRY MATTER DISAPPEARANCE AND MINERAL RELEASE FROM FORAGES INCUBATED IN THE RUMEN, ACID-PEPSIN, AND INTESTINES OF HOLSTEIN COWS

Effect	Degrees of Freedom	Dependent Variables				
		DM	Ca	P	Mg	K
		— Level of significance (P =) —				
Forage (F)	5	001	001	001	001	NS[a]
Cow (C)	2	001	042	042	001	NS
Digestion Site (S)	2	001	001	001	001	NS
F x C	10	002	040	014	030	NS
F x S	10	NS	004	011	001	NS
C x S	4	NS	NS	001	NS	NS
F x C x S	20	NS	NS	025	021	NS
Residual	53					

[a]NS = not significant with P > .05.

TABLE 4-4. LEAST SQUARES MEANS FOR DRY MATTER DISAPPEARANCE FROM SIX FORAGE SPECIES INCUBATED ALONG THE DIGESTIVE TRACT OF HOLSTEIN COWS

Forage	Rumen	SEM	Acid-Pepsin	SEM	Lower Tract	SEM
	%		%		%	
Alfalfa	71.1[a]	.82	75.3[b]	.82	75.8[b]	.82
Rhizoma peanut	64.4[b]	1.14	65.4[a]	1.14	69.3[b]	1.14
Dwarf elephantgrass	58.5[b]	3.40	58.0[b]	3.40	63.5[a]	3.40
Bermudagrass	46.9[a]	1.20	46.1[b]	1.20	47.0[a]	1.20
Bahiagrass	45.0[a]	1.47	45.9[a]	1.47	52.4[b]	1.47
Limpograss	47.0[b]	1.03	47.6[a]	1.03	52.4[b]	1.03

[a,b]Means in the same row with different superscripts are different ($P < .05$).

incubation on DM disappearance from the other five forage species may suggest that their protein wes highly degradable in the rumen. Acid-pepsin or lower tract incubation increased DM disappearance of four of the six forage species beyond that of ruminal disappearance. Unlike BA and L, SE was not digested postruminally. It may have less potentially digestible fiber than BA and L such that all was digested in the rumen or e faster rate of digestion such that all possible digestion was complete in 24 h. If release of certain minerals from forages was dependent upon cell wall digestion, one would expect to see less mineral release from these grasses.

Calcium

Of the four minerals examined, calcium release was affected most by digestion site (Table 4-5). Most calcium was released in the rumen, yet, variability among forages was large ranging from -1.0% for E to 73.9% for A. Acid-pepsin incubation increased calcium release from all forages by an average of 28% (range from 17 to 51%) ($P < .05$). The large effect of acid-pepsin could be related to high ruminal pH experienced by animals in this study. Ruminal pH of these cows varied with time after feeding but remained above 6.5 (Table 4-6). If ruminal pH had been lower, as would be the case for cows consuming high energy diets, additional calcium may have been released in the rumen. As pH of the digesta was decreased, more magnesium aed calcium became associated with the water soluble fractien (Grece et al., 1977). Calcium salts of tannic acid were dissolved at a pH of 5.57 and calcium phosphates dissolve at a pH of 5.79 (Faithfull, 1994). Calcium is known to be attached to the phesphoryl, hydroxy , aed carboxyl groups of

TABLE 4–5. LEAST SQUARES MEANS FOR CALCIUM RELEASE FROM SIX FORAGE SPECIES INCUBATED ALONG THE DIGESTIVE TRACT OF HOLSTEIN COWS

Forage	Rumen		Acid Pepsin		Lower Tract	
	%					
		SEM		SEM		SEM
Alfalfa	73.9[a]	.8	92.2[b]	.8	99.4[a]	.8
Rhizoma peanut	60.7[b]	1.3	74.3[b]	1.3	73.7[b]	1.3
Dwarf elephantgrass	–1.0[a]	15.2	51.9[b]	15.2	59.0[a]	15.2
Bermudagrass	73.8[a]	2.4	86.3[b]	2.4	75.3[a]	2.4
Bahiagrass	43.6[a]	2.0	65.6[b]	2.0	47.0[a]	2.0
Limpograss	63.6[a]	1.0	80.4[b]	1.0	71.7[c]	1.0

[a,b,c]Means in the same row with different superscripts are different ($P < .05$)

TABLE 4-6. EFFECT OF TIME AFTER FEEDING ON RUMINAL pH

Time (h)	Cow 1	Cow 2	Cow 3
	pH		
0	6.66	6.93	6.91
2	6.66	6.77	6.81
4	6.66	6.66	6.76
6	6.52	6.64	6.80
8	6.56	6.60	6.89

hemicellulose. As hemicellulose is susceptible to solubilization in acidic conditions, bound minerals may be released simultaneously. While this may have accounted for some calcium release observed here, no change in DM disappearance (Table 4-4) indicated that little hemicellulose was released. Possibly acid conditions can break bonds holding calcium without freeing whole sugar moieties. Exposure of forages A, P, and E to the intestinal environment resulted in no further release of calcium. However, forages of high fiber content, BE, BA, and L, sequestered calcium during incubation in the lower digestive tract. This difference accounted for the significant forage by digestive site interaction (Table 4-3). Maximal release of calcium from all forages was at least 58% with the majority having release extents over 70%.

Grace et al. (1974) reported that digesta in the ileum of sheep contained 11 to 25% more calcium than digesta entering the duodenum. They observed that digesta from a legume diet increased less in calcium than digesta from a grass diet. The leguminous residues increased less in calcium than the grass residues in the present trial as well (Table 4-5). It appears that calcium exchange capacity of forage cell wall may be a function of forage species, pH, and extent of cell wall digestion.

Calcium in alfalfa was only half as available as calcium from inorganic sources (Ward et al., 1979). This cannot be attributed to lower ruminal or gastro-intestinal release based upon our observations. The calcium in A had the highest ruminal and total tract release of all forages examined (Table 4-5). Using the same alfalfa variety and stage of maturity, Emanuele and Staples (1988a) reported ruminal calcium

release in situ of 59%. This difference (73.9 vs. 59%) was not expected. Emanuele and Staples (1988a) observed that calcium release from these same six forages was slow, reaching maximum release beyond 24 h incubation. Based upon this, calcium release from these forages was expected to be lower in this trial because of the shorter ruminal incubation time. This was true for five of six forages but A was the exception.

Ruminal release of calcium from E was quite variable as indicated by the large SEM (Table 4-5). Extent of ruminal release ranged from -62 to 36%, resulting in a mean release coefficient of -1.0% and a SEM of 14.3%. Microbial contamination likely was not the cause for this variability as magnesium and phosphorus concentrations, which are high in rumen microbes (Durand and Kawashima, 1980, Pitt et al., 1974), responded differently than calcium. In an in situ trial, calcium concentration of E residues increased 20% over the original sample from machine washing only (Emanuele and Staples, 1988a). These samples had not undergone ruminal incubation so microbial contamination was not a factor. McBurney et al. (1983) suggested that cation bonding to cell wall was a function of the number of specific chemical groups exposed rather than cell wall mass. It appears the indigestible cell wall of E contains many negatively charged functional groups, possibly uronic acids and phenolic groups which have a strong affinity for calcium. The cell wall carbohydrates of E need to be quantified before and after ruminal fermentation to determine the cause for the observed binding of calcium by this forage.

Magnesium

The rumen was the major site of magnesium release from these forages (Table 4-7). Ruminal magnesium release was high, 88 to 98% of initial magnesium concentrations. Acid-pepsin incubation increased magnesium release only slightly, although significantly for P, BA, and L ($P < .05$). Magnesium release from A, E, and BE was not increased above ruminal release by acid-pepsin or intestinal incubation. Residues of BA and L increased in magnesium concentration 1 to 5 percentage units as they passed from the duodenum to the feces ($P < .05$). These differences among the forage species in magnesium concentration as they traveled through the digestive tract led to a forage by digestive site interaction ($P < .001$) (Table 4-3). Compared to the other forages, BA had a greater ability to bind calcium and magnesium from the soluble phase in the lower tract. Net secretion of magnesium into the small intestine of forage-fed sheep was 9 to 30% of dietary magnesium intake when organic matter intake (OMI) was 500 or 800 g/d of ryegrass or white clover (Grace et al., 1974). When OMI of white clover was 500 or 800 g/d and OMI of ryegrass was 800 g/d, all of this additional magnesium was reabsorbed in the large intestine. This could explain why the net increase in magnesium concentration observed in the forage residues was much smaller than the net increase of calcium (Table 4-5).

The reticulo-rumen was the main site of magnesium absorption in ruminants (Tomas and Potter, 1976). The availability of released magnesium for absorption will depend on a favorable ruminal environment. ng chain fatty acids in the diet (Jenkins and Palmquist, 1984), high ruminal ammonium levels (Head and Rook, 1957), low ruminal Na/K ratio

TABLE 4-7. LEAST SQUARES MEANS FOR MAGNESIUM RELEASE FROM SIX FORAGE SPECIES INCUBATED ALONG THE DIGESTIVE TRACT OF HOLSTEIN COWS

Forage	Rumen		Acid-Pepsin		Lower Tract	
	%					
		SEM		SEM		SEM
Alfalfa	95.6[a]	.3	96.4[a]	.3	95.6[a]	.3
Rhizoma peanut	97.9[a]	.1	98.4[b]	.1	98.4[b]	.1
Dwarf elephantgrass	95.0[a]	.5	98.2[b]	.5	95.3[a]	.5
Bermudagrass	95.7[a]	.4	96.1[a]	.4	95.2[a]	.4
Bahiagrass	87.7[a]	.6	91.4[b]	.6	88.5[a]	.8
Limpograss	92.7[a]	.3	94.5[b]	.3	93.3[a]	.3

[a,b]Means in the same row with different superscripts are different ($P < .05$).

(Martens and Blume, 1986) and magnesium uptake by rumen microbes (Durand and Kawashima, 1980) reduce both soluble magnesium and magnesium absorption from the rumen. For these reasons magnesium release should not be equated with magnesium absorption

Phosphorus

The rumen was the major site for phosphorus release from these forages (Table 4-8). Ruminal phosphorus release was between 70 and 95% of initial phosphorus concentration. Grass phosphorus release was less than legume phosphorus release (77 vs 93%). High ruminal release coefficients would not be indicative of immediate phosphorus availability to the animal because little phosphorus absorption occurs in the reticulo-rumen (Pfeffer et al., 1970). Total phosphorus release from abomasum plus intestinal sites would be more indicative of phosphorus availability. Acid-pepsin incubation did not increase phosphorus release consistently. Only phosphorus from legumes A and P were susceptible to release by acid-pepsin ($P < .05$). Phosphorus release from bags collected in the feces was high, ranging from 94 to 98% of the total phosphorus in these forages with grasses being lower than legumes. In contrast to calcium and magnesium, phosphorus release increased in the intestines for all forages ($P < .05$). The net absorption of phosphorus from the small intestine was 90 to 140% as a percent of dietary intake in sheep fed ryegrass and clover (Grace et al., 1974). This indicates that the small intestine was capable of absorbing almost all the phosphorus released by these forages which reaches the small intestine in an absorbable form. The type of forage fed and level of phosphorus intake influenced the flow of phosphorus to the duodenum of

TABLE 4-8. LEAST SQUARES MEANS FOR PHOSPHORUS RELEASE FROM SIX FORAGE SPECIES INCUBATED ALONG THE DIGESTIVE TRACT OF HOLSTEIN COWS

Forage	Rumen		Acid-Pepsin		Lower Tract	
	%					
		SEM		SEM		SEM
Alfalfa	92.0[a]	.8	94.8[b]	.8	97.4[c]	.8
Rhizoma peanut	94.6[a]	.2	95.3[b]	.2	97.5[c]	.2
Dwarf elephantgrass	86.0[a]	.8	85.0[a]	.8	94.2[b]	.6
Bermudagrass	72.7[a]	1.2	75.8[b]	1.2	84.4[b]	1.2
Bahiagrass	70.5[a]	.8	72.1[a]	.8	86.2[b]	.6
Limpograss	80.2[a]	.7	81.8[a]	.7	89.9[b]	.7

[a,b] Means in the same row with different superscripts are different ($P < .05$)

sheep (Grace et al., 1974, Leibholz, 1974). Of the six forages, BE and BA diets could result in the lowest flow of phosphorus to the duodenum since they had the lowest phosphorus concentration and release coefficients

Potassium

Potassium release from all forages was greater than 99%, all of which occurred entirely in the rumen (Table 4-9). There were not any differences among the six forages for extent of potassium release. Possible implications of this extensive release of potassium in the rumen are first, that forages high in potassium concentration have greater buffering capacity in the rumen than forages of low potassium concentration, e.g., alfalfa greater than corn silage or grass hays (Jasaitis et al., 1987), and secondly, forages high in potassium concentration could reduce magnesium absorption from the rumen by decreasing the Na:K ratio in the rumen fluid (Wylie et al., 1985; Martens and Blume, 1986).

Supplemental magnesium may be beneficial when forages high in potassium concentration are a major dietary component. The greater buffering capacity of these forages would lessen decreases in ruminal pH. Ruminal pH above 6 reduces the amount of ultrafilterable magnesium available for absorption by dairy cows (Johnson et al., 1988). This reduced amount of potentially absorbable magnesium coupled with reduced magnesium absorption by the ruminal epithelium due to decreasing Na:K ratio could result in a shortage of magnesium in the rumen and blood. This hypothesis was supported by an increase in milk production (5.8%) when buffered diets adequate in magnesium (NRC, 1978) were supplemented

TABLE 4-9. LEAST SQUARES MEANS FOR POTASSIUM RELEASE FROM SIX FORAGE SPECIES INCUBATED ALONG THE DIGESTIVE TRACT OF HOLSTEIN COWS

Forage	Rumen	SEM	Acid-Pepsin	SEM	Lower Tract	SEM
	%					
Alfalfa	99.8[a]	.04	99.8[a]	.04	99.9[a]	.04
Rhizoma peanut	99.8[a]	.57	99.9[a]	.57	98.9[a]	.57
Dwarf elephantgrass	99.9[a]	.01	100.0[a]	.01	100.0[a]	.01
Bermudagrass	99.9[a]	.02	99.9[a]	.02	99.9[a]	.02
Bahiagrass	99.2[b]	.02	99.9[a]	.02	99.9[a]	.02
Limpograss	99.9[a]	.03	99.9[a]	.03	99.8[a]	.03

[a]Means in the same row are not different (P > .05)

with additional magnesium (.48% of DM) (O'Connor et al., 1988).

Discussion

The in situ technique was used to estimate the quantity of a mineral released from six forage species. Of the six forage species, alfalfa is a major forage used in dairy rations. In situ estimates of mineral release could be used to calculate potentially available minerals supplied from alfalfa (Table 4-10). Intake (g/d) of a mineral was multiplied by the percent of the mineral released after acid-pepsin incubation. This release coefficient was selected because absorption of calcium and phosphorus occurs in the small intestine. Calcium and phosphorus release following acid-pepsin incubation would estimate the potentially available mineral reaching the small intestine. Acid-pepsin incubation increased calcium and phosphorus release but did not alter magnesium or potassium release. Not all of this potentially available mineral would be absorbed by the dairy cow. To account for this fact, the potentially available mineral is multiplied by an absorption coefficient. Selecting a value for the absorption coefficient is difficult. Does one select apparent absorption coefficients or true absorption coefficients? Does one apply data collected with sheep to dairy cattle? How does one account for differences in feed intake among different experiments? The following criteria were used in selecting absorption coefficients. First, the assumption that mineral released from forages has the same availability as dietary mineral is probably not true. The dietary mineral contains unreleased or unavailable mineral. This has already been removed from the released mineral fraction of our study. Because of this, average availability estimates

TABLE 4-10. CALCULATING QUANTITY OF MINERALS FROM ALFALFA HAY SUPPLIED TO LACTATING COWS FOR METABOLISM USING SEPARATE RELEASE (TABLES 4-5, 4-7, AND 4-8) AND ABSORPTION COEFFICIENTS (FIELD, 1981) OR A SINGLE ABSORPTION COEFFICIENT (NATIONAL RESEARCH COUNCIL, 1988, OR GREEN ET AL., 1986)

	Ca		Mg		P	
	Field (1981)	NRC (1988)	Field (1981)	Green et al. (1980)	Field (1981)	NRC (1988)
Mineral intake, g/d[a]	102	102	27	27	15	15
Mineral release after acid-pepsin incubation, %[b]	32	—	96	—	95	—
Potentially available mineral, g/d[c]	94	—	26	—	14	—
Absorption coefficient, %[d]	84	38	80	41	75	45
Absorbed mineral, g/d[e]	79	39	21	11	11	7
Gross mineral requirement, g/d[f]	126	126	50	50	80	80
Absorbable mineral requirement, g/d[g]	48	48	21	21	36	36
Mineral requirement supplied by alfalfa, %[h]	164	81	99	53	30	19

[a]7 kg DM alfalfa x mineral concentration of alfalfa (Table 4-1).

[b]Tables 4-5, 4-7, and 4-8

[c]Mineral intake x percent release after acid-pepsin

[d]Field (1981)—based on mineral release; National Research Council (1988) or Greene et al. – based on mineral intake.

[e]Absorption coefficient x potentially available mineral (Field) or mineral intake (NRC or Green et al., 1986).

[f]500 kg cow producing 33 kg milk at 4.0% fat as in NRC, 1988

[g]Gross mineral requirement x NRC or Greene et al. absorption coefficient

[h]Absorbable mineral requirement ÷ absorbed mineral.

of dietary minerals should be lower compared to the released mineral fraction. Yet, little information exists on absorption of the released mineral fraction. Field (1991) stated that absorption of a mineral was equal to the fractional absorption of mineral solubilized in the digestive tract. He defined absorption as A = aI = yMs, where A = absorption (g/d) of daily dietary mineral intake (I), a = fractional (%) absorption of I, y = fractional (%) absorption of mineral solubilized in the digestive tract, and Ms = mineral (g) solubilized in the digestive tract. Field suggests that mineral solubilized in the digestive tract enters the same pool as endogenous mineral secretions. Based upon this assumption he suggested that fractional absorption of solubilized minerals would be 84% for calcium, 75 to 95% for phosphorus, 90 to 90% for potassium and 80 to 90% for magnesium. Of these estimates, only phosphorus was validated by measuring the absorption of endogenous phosphorus over a range of phosphorus intakes with sheep. These absorption coefficients were used with release coefficients generated in this study to estimate g of calcium, magnesium, and phosphorus absorbed by a 500 kg cow producing 33 kg of milk at 4.0% fat (Table 4-10). This was compared with g of mineral absorbed using the absorption coefficients used by the National Research Council (1988) and Agricultural Research Council (1980). The absorption coefficient used for calcium was the National Research Council (1988) estimate. This estimate represents the true absorption of calcium because it accounts for endogenous calcium excretion (Hansard et al., 1957). Yet, this absorption coefficient is for dietary calcium not the released calcium fraction. True absorption coefficient used for dietary magnesium was 41% for Holstein cows (Greene

et al., 1986). The Agricultural Research Council (1980) assumes that soluble sodium and potassium are completely available. The National Research Council (1988) does not estimate potassium availability.

When separate release coefficients can be generated for specific forages of interest and used with general absorption coefficients for released minerals, mineral needs of the cow can be met with an improved efficiency (Table 4-10). This improved efficiency was 102% for calcium, 87% for magnesium, and 58% for phosphorus using alfalfa. Using 6 kg of RE to help meet the mineral needs of this same cow, efficiency was improved 84% for calcium, 87% for magnesium, and 23% for phosphorus.

In conclusion, the mobile dacron bag technique was able to detect differences among forages and sites of digestion for extent of mineral release. The rumen was the major site of release for all minerals studied. Minerals could be ranked based on extent of release in the rumen as follows: potassium > magnesium > phosphorus > calcium. Similar rankings were reported by Rooke et al. (1983) and Whitehead et al. (1985). A significant forage by digestion site interaction occurred for all minerals but potassium. Dwarf elephantgrass appeared to bind calcium in the rumen while BA and L appear to sequester calcium and magnesium in the lower digestive tract. Ranking of forages based on extent of mineral release across minerals examined were A > P > RE > L > E > BA. The differences observed among forages and minerals plus the significant forage by digestion site interactions suggest one should be cautious about making general statements about forage and mineral relationships. It is possible to meet the mineral requirements of ruminants more efficiently by more precise characterization of the

mineral fraction of forages beyond that of simple gross composition If measuring extent of release can be included as part of a routine lab analysis, a ration may be balanced more accurately for absorbable mineral This would be one more step to making balancing a ration more of a science and less of an art

CHAPTER V
EFFECTS OF READILY FERMENTABLE CARBOHYDRATES AND RUMINAL pH ON RUMINAL MINERAL RELEASE AND FIBER DISAPPEARANCE FROM ALFALFA HAY

Introduction

Dairy cows often are fed diets of forage to concentrate ratios of 60:40 to 40:60 during early lactation. Buffers, such as sodium bicarbonate, are used to raise ruminal pH, milk fat percentage and milk yield (Erdman, 1988). Readily fermentable carbohydrates not only lower reminal pH, but increase VFA production. Both factors influence magnesium utilization. Lowering ruminal pH increases the amount of ultra-filterable magnesium in the rumen of lactating cows indicating decreased absorption (Johnson et al., 1988). Increasing VFA concentration by supplementing readily fermentable carbohydrates to an alfalfa hay diet increased apparent absorption of magnesium, calcium and phosphorus by sheep (Rayssequier and Poncet, 1980). The greatest increase was in ruminal calcium and magnesium absorption. Fecal calcium excretion was decreasnd and apparent absorption of calcium increased, when lambs fed 100% bromegrass were switched to diets contening 50% concentrate (Harmon and Britton, 1983). Urinary calcium excretion was increased and calcium retention decreased in these sama lambs (Harmon and Britton, 1983). In the sama study, fecal magnesium and phosphorus excretion were decreased and magnesium and phosphores retention increased when lambs were switched from an all forage diet to a 50:50

forage to concentrate ratio diet. Giduck and Fontenot (1987) supplemented sheep fed orchardgrass hay with 450 g of either glucose, sucrose, lactose or starch. The supplementation of the forage diet with readily fermentable carbohydrates increased apparent magnesium absorption and retention. Calcium retention was decreased when orchardgrass was supplemented with sucrose or lactose because of increased urinary excretion of calcium (Giduck and Fontenot, 1987). Apparent potassium absorption was decreased slightly by carbohydrate supplementation but phosphorus excretion was not affected in this study.

In all these studies, ruminal pH was altered while simultaneously increasing the energy concentration of the diet. Both pH and energy concentration appeared to alter mineral absorption but it is necessary to separate the two effects. The objectives for this study were to determine:

1. the effect of decreasing ruminal pH on in situ fiber disappearance and mineral release in the rumen,
2. the effect of dietary energy supplementation on in situ fiber disappearance and mineral release independent of the effect of pH,
3. if decreasing ruminal pH or increasing energy concentration of the diet alters the concentration of calcium, magnesium, phosphorus, potassium, copper or zinc in the ruminal liquid phase and solid phase of ruminal digesta, and affects ruminal outflow (g/d) of these minerals.

Materials and Methods

Four nonlactating mature Holstein animals fitted with ruminal cannulae were arranged in a 4 x 4 Latin square design. The four dietary treatments were:

1. control (C)—chopped alfalfa hay plus ruminal infusion of 3 L of distilled water, containing mineral supplement,
2. acid (A) – chopped alfalfa hay plus ruminal infusion of 3 L of 1 N HCl, containing mineral supplement,
3. energy (E) – chopped alfalfa hay and ground corn plus ruminal infusion of 3 L of distilled water containing mineral supplement, and
4. energy buffered (EB) – chopped alfalfa hay and ground corn plus ruminal infusion of 3 L of distilled water containing sodium bicarbonate plus mineral supplement.

Feedstuffs were fed once daily at 0800 h to restricted amounts. Hay DM intake was 1.2% of body weight, whereas ground corn DM intake was .8% of body weight resulting in a forage to concentrate ratio of 60:40. The infusion solutions contained different amounts of NaCl, NH_4Cl, KH_2PO_4, and $MgSO_4$, so that an animal received similar quantities of magnesium, potassium, sodium and chloride on each treatment. Solutions were infused into the rumen via Haake Buchler Multistatic pumps (Haake Buchler Instruments, Inc., Saddle Brook, NJ 07662). All infusions lasted 4 h beginning 30 min after feeding. Chemical and ingredient composition of the dietary treatments are in Table 5-1. Quantities of minerals provided by treatments are in Table 5-2. Amounts of sodium, potassium, chloride, calcium, and magnesium (g/d) supplied to the

TABLE 5-1 CHEMICAL COMPOSITION OF FEEDSTUFFS AND DAILY INTAKE OF DIETARY INGREDIENTS AND INFUSED COMPOUNDS

Chemical composition

Feedstuff	CP	NDF	Na[a]	K	Ca	Mg	P	Cl[a]	Cu	Zn
	% DM								ppm	
Alfalfa hay	19.2	51.7	.12	2.74	1.46	.32	.25	.38	7.6	19.6
Corn	10.0	9.0[a]	.03	.40	.07	.13	.33	.05	2.0	21.3

Ingredient intake

Ingredient	Treatment			
	Control	Acid	Energy	Energy Buffer
	g/d			
Alfalfa hay	5,000	5,000	5,000	5,000
Corn	0	0	3,400	3,400
NaCl	63	63	63	0
NH_4Cl	161	0	161	218
KH_2PO_4	45	45	0	0
$MgSO_4$	20	20	0	0
HCl	0	294	0	0
$NaHCO_3$	0	0	0	90

[a]National Research Council, 1988; tabular values, all other values are determined by laboratory analysis

TABLE 9-2. ESTIMATED QUANTITY OF EIGHT MINERALS PROVIDED BY DIET AND INFUSATES TO RUMINALLY-INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

	Treatments					
	Control			Acid		
Mineral	Hay	Infusion	Total	Hay	Infusion	Total
	g/d					
Sodium	7	25	32	7	25	32
Potassium	137	13	150	137	13	150
Chloride	16	105	125	16	106	125
Calcium	76	0	76	76	0	76
Phosphorus	14	10	24	14	10	24
Magnesium	16	4	26	16	4	20
	mg/d					
Copper	36	0	36	38	0	38
Zinc	100	6	106	100	0	100

	Treatments						
	Energy				Energy Buffer			
Mineral	Hay	Corn	Infusion	Total	Hay	Corn	Infusion	Total
	g/d							
Sodium	7	1	25	33	7	1	25	33
Potassium	137	14	0	151	137	14	0	151
Chloride	16	1 7	104	125	16	1-7	104	125
Calcium	79	2 6	0	61.6	79	2-6	0	61.6
Phosphorus	14	11	25	25	14	11	0	25
Magnesium	16	4 4	0	20 4	16	4.4	0	20-4
	mg/d							
Copper	36	7	0	45	38	7	0	45
Zinc	100	72	0	172	100	72	0	172

animals were in excess of the National Research Council (1988), recommendations for dry pregnant cows, whereas phosphorus intake was equal to the daily requirement (National Research Council, 1988). The dietary treatments as formulated were deficient in copper and zinc for dry pregnant cows. Trace mineralized salt was supplied to all animals during adjustment periods but was withdrawn from the diet during sample collection periods so that all dietary copper and zinc would be from feedstuffs.

Each experimental period was 18 d, composed of 7 d of adjustment followed by 11 d of sampling. The sampling schedule for each period was in situ incubation on d 9 to 10, followed by sampling of ruminal liquid for pH, VFA and ammonia concentration on d 12. Chromium concentration was measured also on these samples to estimate liquid passage rate. On d 13, liquid and solid digesta was removed from the rumen for mineral analysis. Fecal sampling for solids passage rate determination occurred on d 14 to 17. Manual emptying and weighing of ruminal contents occurred on d 18.

The in situ study was performed using 15 x 25 cm dacron bags with mean pore size of 53 μm (Marvelaire white, Erlanger, Blumgart and Co., Inc., New York, NY). Alfalfa hay was ground through a 2 mm Wiley mill screen. Fifteen g were weighed into each dacron bag resulting in a substrate weight to bag surface area ratio of 20 mg/cm^2. Bags were placed in the rumen in time sequence with all bags removed simultaneously after 72 h. Incubation times were 0, 3, 6, 12, 18, 24, 36 and 72 h. After incubation, dacron bags were placed in an ice water bath for 15 min, then washed in a commercial washing machine without

detergent. Bags thee were washed with distilled water 15 to 20 times until effluent from bags was clear. Bags were dried at 55°C and weighed hot. One replicate set of bags was used for analysis of NDF. Neetral detergent fiber was measered withoet decalin or sodium sulphite (Golding et al., 1985). The second bag replicate residues were analyzed for mineral concentration. Bag residues were ashed at 550°C for 2-5 h. Ashed samples were solubilized in 15 ml of 3 N HCl and boiled gently for 10 min on a hot plate (Association of Official Analytical Chemists, 1980). Calcium, magnesium, potassium, copper and zinc were determined by atomic absorption spectrophotometry (Model 5000, Perkin-Elmer, Inc., Norwalk, CT). Phosphorus was measured colorimetrically (Harris and Popat, 1954).

The model for kinetics of DM and NDF disappearance and mineral release was as described by Mertens and Loften (1980):

$$R = D_o e^{-k(t-L)} + U$$

when $t > L$ and $R = D_o + U$ when $0 < t < L$, and where:

R = g or mg of DM, NDF, calcium, magnesium, phosphorus, copper or zinc in residue at incubation time = t,

D_o = g or mg of slowly released fraction,

k = release rate constant,

L = discrete lag time in h, and

U = residue at maximum extent of release in g for calcium, magnesium, and phosphorus and mg for copper and zinc.

Release rate constaets were calculated with the nonlinear model procedure of SAS using Marquardt's methnd (SAS Users Guide, 1985).

On d 12, samples of ruminal liquid were taken via ruminal fistula by suction strainer at 1, 2, 3.5, 5.0, 6.5, 8.0, 9.5, 11 and 12.5 h after feeding. The pH was determined immediately after sampling with glass electrode. Ruminal fluid pH data were converted to hydrogen ion concentration for determination of treatment differences (Murphy, 1982). After measuring fluid pH, samples were acidified with 50% H_2SO_4 so that pH was below 2.5. Samples were centrifuged at 2,792 x g for 15 min. Supernatant was frozen at -30°C in plastic vials until analyses for ammonia and VFA concentrations. Prior to analysis, samples were thawed and centrifuged a second time at 17,300 x g for 15 min. Ammonia concentrations were determined by the method of Chaney and Marbach (1962). Volatile fatty acids were determined (Erwin et al., 1961) by gas chromatography using a carbowax column, nitrogen gas carrier and temperature of 200°C (Model 5890 A with integrator, Hewlett-Packard Co., Avendale, PA).

On d 12, 2 h after feeding, ruminal liquid dilution rates were determined by dosing a 1 L solution containing 2.9 g of chromium-ethylenediaminetetraacetic acid (Cr-EDTA) via fistula (Binnetts et al., 1968). Samples of ruminal fluid were taken by suction strainer from five locations in the ventral sac of the rumen at 1.5, 3.0, 4.5, 6.0, 7.5, 9.0 and 10.5 h after dosing with Cr-EDTA. Samples for chromium analyses were processed and stored in the same manner as ammonia samples. Chromium concentration in the rumminal fluid was measured by atomic absorption spectrophotometry. Rate of liquid passage from the rumen was calculated by linear regression of the natural logarithm of chromium concentration on time after dosing.

On d 13, samples of liquid were collected from the ventral sac of the rumen over time by suction strainer for mineral analyses. Sampling times were 1, 2, 3.5, 5, 6.5, 9, 9.5, 11, and 12.5 h after feeding. Liquid samples were centrifuged at 2 792 x g for 15 min. Supernatant was frozen in plastic vials at -30°C. The supernatant was thawed and centrifuged at 20,000 x g for 30 min prior to analysis (Grace et al., 1988). Digesta was collected by grab sampling from five sites in the rumen. Times of sampling were 2, 3.5, 6.5, 9.5 and 12.5 h after feeding. Digesta samples were mixed together in a plastic bucket, strained through eight layers of cheese cloth, placed in plastic bottles, and frozen at -30°C. Upon thawing, digesta samples were placed into 15 x 25 cm dacron bags and washed 15 to 20 times with distilled water, until effluent from the bags was clear. Ruminal fluid samples were diluted with 1 N HCl for potassium, phosphorus, copper and zinc analysis. Ruminal fluid samples were diluted with 1% lanthanum chloride for calcium and magnesium analysis. Solid digesta samples (2 g DM) were ashed at 550°C for 2.5 h and solubilized in 3 N HCl.

To determine rate of passage of solids, 5 kg of air-dried hay was labeled by soaking in 50 L of ytterbium chloride ($YbCl_3$) solution for 24 h. The soaking solution contained 2.6 g $YbCl_3$ per L of distilled water (Goetsch and Galyean, 1983). After 24 h, hay was soaked in distilled water every 30 min for 3 h, drained on a metal screen, and dried at 55°C for 72 h. Yield of labeled hay was 2.5 to 3 kg. A new batch of hay was marked for each period, and a sample retained for ytterbium analysis. On d 14, animals were fed 600 g of air-dried ytterbium-labeled chopped alfalfa hay prior to the morning feeding.

Any hay not consumed after 20 min was placed into the rumen and mixed into the digesta. Fecal grab samples were collected 9, 12, 18, 20, 24, 28, 32, 36, 42, 48, 54, 60, 72, 84, 96 h after feeding the labeled hay. Fecal samples were frozen immediately after collection. Prior to ytterbium analysis, fecal samples were dried at 55°C and ground through a 2 mm screen on a Wiley mill. Feed and fecal samples were analyzed for ytterbium by atomic absorption spectrophotometry following extraction with diethylenetriaminepentaacetic acid (DPTA) (Karimi et al., 1997). Rate of solids passage was calculated by regression of the natural logarithm of ytterbium concentration on time after peak of ytterbium concentration in feces was reached.

To determine ruminal volumes of solids and liquids, the ruminal contents from each animal were removed manually prior to the morning feeding. Contents were weighed, mixed, and a sample taken for DM analysis (55°C for 72 h.). Ruminal contents were returned to each animal.

Statistical analysis was performed using the GLM procedure of SAS (SAS, 1985). Single degree of freedom contrasts for treatments were performed using least squares means. Contrasts tested were 1) the effect of supplemental energy (E plus EB vs. C plus A); 2) the effect of pH at low energy concentration of the diet (C vs. A); and 3) the effect of pH at high energy concentration of the diet (E vs. EB). The model contained period, cow and treatment and residual as sources of variation. The F statistic for the preplanned contrasts had 1 df for the contrast mean square and 3 df for the error mean square.

Results and Discussion

Effect of diet and infusates on ruminal pH, VFA concentrations, and ammonia concentrations is in Table 5-3. Infusion of 1 N HCl did not decrease ruminal pH below control values (P = .121). Although a wide numerical difference existed (6.1 vs 6.8), one animal was unresponsive to HCl infusion. Supplementation of alfalfa hay with corn reduced ruminal pH (P = .010). Addition of $NaHCO_3$ to E increased ruminal pH (P < .006) above E alone. Total millimoles of VFA were highest with energy supplementation (P = .010). Elevation of the pH by $NaHCO_3$ decreased total VFA concentration (P = .054) as seen previously (Staples et al., 1988). Energy supplementation decreased the molar percentage of acetic acid (P = .020), increased the molar percentage of butyric acid (P = .058) and valeric acid (P = .044) and increased slightly though not significantly the molar percentage of propionic acid (P = .10). The overall effect was a decrease in the acetate-to-propionate ratio when energy was supplemented (E,EB vs C,A) (3.3 vs 2.6) (P = .060). Oshio et al., (1987) reported similar VFA patterns in Holstein heifers fed an orchardgrass hay diet supplemented with rolled barley. When the forage to concentrate ratio was switched from 100:0 to 75:25 or 50:50, total mM of VFA was increased, molar percent acetic acid decreased and molar percent butyric acid increased. It was not until forage to concentrate ratio was 25:75 that molar percentage of propionic acid increased above the concentration when the forage to concentrate ratio was 100:0.

Acid infusion in the rumen resulted in no changes in the molar proportions of VFA compared with C. Likewise, Gilduck et al., (1988) reported also that intraruminal HCl infusion decreased ruminal pH but

TABLE 5-3. LEAST SQUARES MEANS FOR RUMINAL PH AND CONCENTRATIONS OF AMMONIA AND VOLATILE FATTY ACIDS (VFA) OF RUMINALLY INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

	Treatments					Contrasts		
Measurement	Control	Acid	Energy	Energy Buffer	SEM	Energy & Energy Buffer vs. Others	Acid vs Control	Energy vs Energy Buffer
						(P =)		
pH	6.8	6.1	5.6	6.2	.24	.010	.121	.006
Ammonia, mg %	17.6	10.4	14.1	15.8	3.21	.772	.278	.721
VFA								
Total mM/L	71.1	77.5	93.1	83.0	3.75	.010	.227	.054
Acetic[a]	71.1	67.3	62.5	64.6	1.18	.020	.196	.324
Propionic	19.3	23.4	26.4	24.7	2.09	.100	.296	.559
Butyric	6.9	6.8	8.3	8.3	.29	.058	.881	.997
Isobytric	.7	.6	.6	.6	.04	.323	.516	.777
2-Methyl butyrate	.5	.4	.5	.4	.03	.119	.212	.088
Valerate	.9	1.0	1.2	1.0	.06	.044	.389	.130
Isovalerate	.5	.4	.4	.3	.03	.252	.619	.791
Acetate/propionate	3.6	3.0	2.5	2.7	.24	.060	.281	.705

[a] Individual VFA'S expressed as mmol/100 mmol total VFA

did not altar molar proportions of individual VFA compared with water infusion. Mould and Orskov (1983) reported that sheep fed chopped hay and infused with mineral acids intraruminally did not have altered proportions of butyric acid, but proportions of acetic acid were increased. The overall effect was a small increase in the acetate to propionate ratio. Molar percentage of VFA was not different among animals infused with $NaHCO_3$ (EB) compared with those receiving E. This often has been the case with alfalfa hay diets (Rogers et al., 1985)

Ruminal ammonia concentration was not different among treatments (Table 5-3). Giduck and Fontanot (1987) found that carbohydrate addition to a chopped orchardgrass hay diet fed to sheep depressed ruminal ammonia levels compared with no carbohydrate supplementation. Acid infusion into the rumen has increased ruminal ammonia concentration compared with water infusion in sheep fed orchardgrass (Giduck et al., 1988). The lack of a response in ruminal ammonia concentration when energy was supplemented in the diet or acid infused could be caused by two factors. First, diets formulated for this experiment were based on alfalfa hay. Second, infusates for C, E and EB contained ammonium chloride. Ammonium chloride in the infusates likely increased ruminal ammonia concentrations. As a result, all diets contained high amounts of nonprotein nitrogen, and nitrogen concentration was in excess of the animal's requirement.

There was no affect of energy concentration on extent of NDF disappearance or mineral release from chopped alfalfa hay incubated in situ (Table 5-4). Acid infusions did not affect extent of NDF

TABLE 5-4 LEAST SQUARES MEANS FOR EXTENT OF OBY MATTER AND NDF DISAPPEARANCE AND MINERAL RELEASE FROM ALFALFA INCUBATED IN RUMINALLY-INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

	Treatment				
Variable	Control	Acid	Energy	Energy Buffer	SEM
	%				
DM	82.1	80.7	79.4	79.8	39
NDF	68.3	65.0	65.0	64.7	1.30
Calcium	86.3	88.9	87.2	86.5	59
Magnesium	94.0	94.5	94.5	94.3	12
Potassium	99.9	99.9	99.9	99.9	.01
Phosphorus	86.5	92.5	87.9	89.1	1.28
Copper	85.9	89.0	87.1	87.0	.64
Zinc	78.4	70.6	76.9	78.4	1.86

	Contrasts		
	Energy and Energy Buffer vs. Others	Acid vs Control	Energy vs Energy Buffer
Variable	(P =)		
DM	.001	.015	.514
NDF	.158	.141	.834
Calcium	.557	.296	.678
Magnesium	.784	.554	.809
Potassium	.340	.625	.784
Phosphorus	.247	.017	.301
Copper	.798	.283	.975
Zinc	.475	.310	.790

disappearance or calcium, magnesium, potassium, copper or zinc release from chopped alfalfa hay incubated in situ. Acid infusion increased extent of phosphorus release (P = .017), six percentage units over C (Table 5-4). Phosphorus concentration in ruminal fluid was increased from 44 to 62 mg/dl when 34 M HCl was infused into the rumen of sheep consuming orchardgrass hay (Giduck et al., 1989). Sheep receiving an unbuffered VFA ruminal infusate had a lower ruminal pH (6.4 vs. 7.0) and a greater phosphorus concentration (58 vs. 43 mg/dl) in ruminal fluid than sheep infused with a buffered VFA solution. Extent of in situ DM disappearance was decreased by energy supplementation (P = .001) and acid infusion (P = .015) compared with C. This depression in DM disappearance cannot be attributed to a depression in NDF disappearance as neither energy (P = .158) nor acid infusion (P = .141) decreased NDF disappearance. However, this does not rule out a particular fiber fraction such as cellulose as the nutrient of depressed disappearance. These findings agree with earlier studies. Miller and Muntifering (1985) examined the effect of forage to concentrate ratios on the kinetics of forage fiber disappearance in situ. They used a 5 x 5 Latin square design with the following forage to concentrate ratios: 100:0, 80:20, 60:40, 40:60 and 20:80. Forage was ground fescue hay, and concentrate was ground corn. Four g of ground fescue hay were placed into 7.5 x 15 cm nylon bags. Apparent extent of fiber disappearance was not reduced until the forage to concentrate ratio was 40:60. Mould et al. (1983) supplemented a chopped hay diet with barley and measured in situ DM disappearance with sheep. Treatments were five forage to concentrate ratios: 100:0, 75:25, 50:50, 25:75, and 0:100. In situ DM

disappearance of the chopped hay was depressed as the quantity of barley in the diet increased. This depression was not significant until forage to concentrate ratio was 50:50. Staples et al. (1988) reported extent of DM and cellulose disappearance of corn silage measured in situ was not different between an acidogenic basal diet and a $NaHCO_3$-buffered diet, but buffers improved cellulose disappearance of bermudagrass hay. Acidic conditions within the rumen can depress disappearance of forage DM and fiber constituents. However, release of minerals was not inhibited by acidic conditions, and in the case of phosphorus, an improvement was observed. In this trial, extent of mineral release was high, greater than 70% (Table 5-4). Extent of release, averaged across treatments, was 87.2% for calcium, 94.3% for magnesium, 99.9% for potassium, 85% for phosphorus, 87.3% for copper, and 76.1% for zinc. These extents of release agree for the most part with those obtained with alfalfa hay using smaller (17 x 9 cm) dacron bags (Emanuele and Staples, 1988a). A difference between the two experiments was the extent of calcium release. An increase in calcium release of 28 percentage units was observed in this experiment compared to Emanuele and Staples (1988). Alfalfa used in the current study was less mature.

Rate of DM and NDF disappearance and magnesium, phosphorus, copper and zinc release from alfalfa hay was not affected by any of the four treatments, C, A, E, or E8 (Table 5-5). Miller and Mantiferiag (1985), Hertens and Loften (1980), Maaid et al. (1983), and Staples et al. (1988) reported no effect of energy or pH on rate of DM, NDF, or cellulose disappearance. This was true as long as forage to concentrate ratio was 50:50 or greater and ruminal pH was between 6.7 and 6.6.

TABLE 5-5. LEASE SQUARES MEANS FOR RATE CONSTANTS FOR DM AND NDF DISAPPEARANCE AND MINERAL RELEASE FROM ALFALFA INCUBATED IN RUMINALLY INFUSED NOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

	Treatment				
Measurement	Control	Acid	Energy	Energy Buffer	SEM
	h^{-1}				
DM	.083	.077	.091	.076	.009
NDF	.072	.056	.064	.069	.004
Calcium	.314	.102	.163	.115	.039
Magnesium	.239	.126	.235	.211	.069
Phosphorus	.152	.129	.120	.085	.062
Copper	.164	.079	.057	.107	.052
Zinc	.309	.110	.164	.113	.090

	Contrasts		
Measurement	Energy and Energy Buffer vs. Others	Acid vs. Control	Energy vs. Energy Buffer
	(P =)		
DM	.652	.526	.198
NDF	.773	.379	.730
Calcium	.200	.071	.463
Magnesium	.637	.492	.640
Phosphorus	.629	.900	.634
Copper	.534	.473	.647
Zinc	.425	.202	.505

Rate of calcium release from alfalfa hay tended to be slower by sheep receiving A compared to C (P = .071).

Concentration of calcium in the liquid phase of the ruminal digesta was increased by energy supplementation (P = .038) (Table 5-6). Acid infusion tended to increase calcium concentration in the liquid phase as well (P = .185). Others have reported similar results (Giduck et al., 1988). Grace et al. (1977) reported more calcium to be associated with the water soluble fraction as digesta pH decreased. Only calcium concentration was affected by both pH and energy concentration. Animals receiving treatments A and EB had similar ruminal pH while being different in energy content. Animals with the lowest pH (E) had the highest ruminal calcium concentration, while those with the highest pH (C) had the lowest ruminal calcium concentration. As ruminal pH dropped, calcium concentration in the liquid phase increased. This suggests that ruminal pH was an important factor affecting calcium concentration in the liquid phase.

Energy supplementation nor acid infusion altered the concentration of magnesium, phosphorus, copper or zinc in the liquid phase. The lack of an effect of acid on ruminal magnesium concentration was surprising. Grace et al. (1977), Garton (1951) and Johnson et al. (1988) all reported increases in liquid phase magnesium as ruminal pH decreased. Giduck et al. (1988) reported that only HCl infusion increased ruminal magnesium concentration above control values while supplemental carbohydrate infusions did not. There may be a pH and energy interaction influencing magnesium concentration. When pH and energy were low (A),

TABLE 5-6 LEAST SQUARES MEANS FOR RUMINAL CONCENTRATIONS OF MINERALS IN THE LIQUID PHASE OF THE DIGESTA OF RUMINALLY INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

	Treatment				
Mineral	Control	Acid	Energy	Energy Buffer	SEM
	ppm				
Calcium	147.1	253.4	328.1	252.3	36.8
Magnesium	100.9	163.3	138.8	128.5	11.1
Potassium	1112.1	1855.3	1694.5	1453.3	68.6
Phosphorus	92.8	147.9	153.5	121.6	7.5
Copper	.26	.23	.27	.29	.04
Zinc	1.21	1.15	1.21	1.24	.27

	Contrasts		
Mineral	Energy and Energy Buffer vs. Others	Acid vs Control	Energy vs Energy Buffer
	(P =)		
Calcium	.038	.105	.125
Magnesium	.934	.138	.696
Potassium	.188	.076	.225
Phosphorus	.412	.194	.302
Copper	.251	.504	.584
Zinc	.578	.720	.807

magnesium concentration in the liquid phase was 163 ppm. If pH is low and energy high (E), magnesium concentration was 138 ppm. Possibly at the higher ruminel energy concentration there is greater microbial growth and metabolism of soluble magnesium.

Acid infusion tended to increase ruminel potassium concentration (P = .076) compared with C. Of the minerals studied, potassium had the highest concentration in the ruminal liquid phase of the digesta, followed by calcium, magnesium, phosphorus, zinc, and copper. There were not any differences between E and EB in concentration of minerals in the liquid phase.

Concentration of calcium (P = .003), magnesium (P = .001), and phosphorus (P = .084) was decreased in the solid phase of the ruminel digesta when animals were supplemented with corn (Table 5-7). Copper concentration in the solid phase of the ruminel digesta tended to be higher with these same treatments (P = .081). Energy had no effect on potassium or zinc concentration in the solid digesta. The decrease in calcium concentration in the solid phase of the digesta by energy supplementation was reflected by an increase in its concentration in the liquid phase. Magnesium concentration did not increase in the liquid phase even though its concentration decreased in the solid phase. Three possibilities exist for this lack of agreement: rapid absorption from the liquid phase into the blood, uptake of these elements by ruminal protozoa and bacteria, and the formation of magnesium ammonium phosphate (Mayland and Grunes, 1978). Rapid absorption was a possibility, because 25% of the daily magnesium intake making up C and A was supplied by magnesium sulfate. Magnesium sulfate is absorbed readily by ruminants

TABLE 5-7. LEAST SQUARES MEANS FOR RUMINAL CONCENTRATION OF MINERALS IN THE SOLID PHASE OF THE DIGESTA OF RUMINALLY INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

	Treatment				
Mineral	Control	Acid	Energy	Energy Buffer	SEM
	ppm				
Calcium	9274	8550	7802	8103	342
Magnesium	951	818	667	790	47
Potassium	231	217	175	333	34
Phosphorus	1122	851	736	662	162
Copper	4.7	5.1	4.0	5.4	.14
Zinc	21.0	20.6	20.1	21.3	.79

	Contrasts		
Mineral	Energy and Energy Buffer vs. Others	Acid vs. Control	Energy vs. Energy Buffer
	(P =)		
Calcium	.003	.207	.508
Magnesium	.001	.117	.071
Potassium	.426	.832	.003
Phosphorus	.084	.368	.758
Copper	.081	.174	.010
Zinc	.932	.776	.262

compared with magnesium from forages (Peeler, 1972). The microbial fraction would be removed from the liquid phase by centrifugation at 20,000 x g (Grace et al., 1988). It would be removed partially from the solid phase by the washing method employed during this trial. Because of the methods used to prepare samples, the contribution of the microbial fraction to the liquid and solid phases of the ruminal digesta was not estimated. The formation of magnesium ammonium phosphate is the least likely alternative. Hayland and Grunes (1979) reported that this compound is difficult to find in the digestive tract and when it is located, it has been found in the intestinal region.

Acid infusions in the rumen did not affect the concentration of minerals in the solid phase of the digesta (Table 5-7). However, at high energy intake, pH appeared to have an effect on mineral concentration. When energy concentration was equal (E vs. ER), magnesium ($P = .071$), potassium ($P = .003$), and copper ($P = .010$) concentrations in the solid phase were decreased at the lower pH. In the liquid phase, when energy is high and pH low (E), mineral concentration was greatest for calcium, potassium, and phosphorus. In the solid phase of the digesta, this same combination (high energy, low pH) caused the lowest concentration of calcium and magnesium. A factorial experiment should be performed to determine if there is a significant interaction between the effect of pH and energy on mineral concentration in the liquid and solid phase of ruminal digesta. Ranking of minerals in the solid phase of the digesta from highest to lowest concentration was calcium > phosphorus > magnesium > potassium > zinc > copper.

Increasing daily DM intake with ground corn (E and E8) tended to increase liquid dilution rate (P = .108) and liquid outflow per day (P = .076) (Table 5-8). Solids passage kinetics were not different among treatments. Total weights of ruminal contents were 25.5, 38.6, 34.1, and 30.2 kg for animals receiving C, A, E, and E8 treatments.

Daily ruminal calcium outflow from the liquid phase of the digesta was increased by energy supplementation (P = .014) and by A (P = .09) (Table 5-9). Energy (E) and E8 were not different in liquid phase calcium outflow. This suggests that the effect of pH (5.6 vs 6.2) was moderated by energy concentration of the diet. There appears to be a pH by energy interaction for calcium outflow in the liquid phase because calcium concentration was different between A and C but similar for E and E8. Total calcium outflow from the rumen was increased by A compared with C (P = .052) and tended to be higher with energy supplementation (P = .117). This was the result of greater ruminal liquid outflow (kg/d) for E and E8 (Table 5-8). Liquid outflow of calcium as a percent of total ruminal calcium outflow tended to be increased (55.4 vs 39.8%) by energy supplementation (P = .104). More of the calcium leaving the rumen would be available for absorption when corn was fed with alfalfa than when alfalfa was fed alone. Giduck et al. (1986) reported that infusion of glucose into the rumen of sheep fed orchardgrass hay increased calcium excretion in the urine over water infusion. Still, 42 to 64% of ruminal calcium was in the solid phase of the digesta, indicating the potential for further calcium release from this phase in the abomasum. An acid-pepsin environment routinely released 18 to 20 percentage units more calcium from forages beyond that

TABLE 5-8 LEAST SQUARES MEANS FOR RUMINAL LIQUID AND SOLID PHASE WEIGHT PASSAGE RATE AND DAILY OUTFLOW OF RUMINALLY INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

	Treatment				
Measurement	Control	Acid	Energy	Energy Buffer	SEM
Liquid dilution rate, h^{-1}	099	086	107	134	018
Solids passage rate, h^{-1}	036	038	037	033	006
Liquid weight, kg	23.9	35 2	30 7	27 6	2.4
Solids weight, kg	1.6	3.4	3.4	2.6	.4
Liquid outflow, kg/d	58	73	79	89	7 5
Solids outflow kg/d	1 4	3.3	3 0	2 1	.6

	Contrasts		
Measurement	Energy and Energy Buffer vs Others	Acid vs. Control	Energy vs Energy Buffer
	(P =)		
Liquid dilution rate, h^{-1}	108	607	218
Solids passage rate, h^{-1}	690	806	539
Liquid weight, kg	894	466	466
Solids weight, kg	253	245	245
Liquid outflow, kg/d	076	313	408
Solids outflow, kg/d	704	189	359

TABLE 5-9. LEAST SQUARE MEANS FOR DAILY RUMINAL CALCIUM OUTFLOW OF RUMINALLY INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

	Treatments					Contrasts		
Measurement	Control	Acid	Energy	Energy Buffer	SEM	Energy and Energy Buffer vs. Others	Acid vs. Control	Energy vs. Energy Buffer
						P =		
Intake, g/d	77.3	77.8	80.8	80.7	1.3	.001	.329	.851
Liquid outflow, g/d	5.8	19.3	24.4	22.4	2.3	.014	.09	.551
Solids outflow, g/d	14.2	27.7	23.5	17.4	5.1	.918	.222	.432
Total outflow, g/d	20.0	47.0	47.9	39.8	5.1	.117	.052	.312
Liquid outflow as % of total outflow	30.3	43.2	52.7	58.1	7.3	.104	.819	.617

released in the rumen (Emanuele and Staples, 1988b).

Total magnesium outflow from the rumen was increased by A (P = .052) compared with C (Table 5-10). This increase was 3.6 g/d, but did not result in significant differences in apparent magnesium absorption from the rumen. There were no other treatment effects on magnesium outflow and absorption from the rumen. Magnesium found in the liquid phase of the ruminal digesta accounted for 83.3 to 85.8% of the total magnesium leaving the rumen. This suggests that additional magnesium release from the solid phase would be small. These data suggest that emphasis should be placed on maximizing magnesium absorption from the liquid phase of the digesta. Apparent magnesium absorption was not increased by energy supplementation. Magnesium absorption was not consistently increased by dietary additions of sucrose or starch (Hemse and Mayland, 1976), and glucose (Madsen et al., 1976). Giduck and Fontenot (1987) observed that supplemental dietary energy in the form of glucose, sucrose, lactose and starch increased apparent absorption of magnesium by sheep fed orchardgrass hay. Giduck et al. (1988) reported that 500 ml of 40% glucose solution infused into the rumen of sheep increased magnesium absorption (P < .05) as measured in metabolism studies.

Total potassium outflow from the rumen was increased by both energy supplementation (P = .029) and acid infusion (P = .034) (Table 5-11). Potassium outflow via the solid phase of the digesta was not affected by treatment, while both energy supplementation (P = .029) and acid infusion (P = .035) increased ruminal potassium outflow in the liquid phase. Therefore, differences observed in total potassium

TABLE 5-10 LEAST SQUARE MEANS FOR DAILY RUMINAL MAGNESIUM OUTFLOW ARD APPARENT ABSORPTION OF RUMINALLY-INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

	Treatments					Contrasts		
Measurement	Control	Acid	Energy	Energy Buffer	SEM	Energy and Energy Buffer vs. Others	Acid vs. Control	Energy vs. Energy Buffer
						P =		
Intake, g/d	21.2	21 3	21.5	21.6	.08	.039	.329	.551
Liquid outflow, g/d	9 6	12 0	11 1	11 4	.75	.580	.153	.758
Solids outflow, g/d	1 5	2 6	2 0	1 7	.48	.524	.272	.689
Total outflow, g/d	11 1	14 7	13.1	13.1	.66	.781	.052	.955
Apparent absorption, %	45 2	34 1	35 4	38 7	4 0	.505	.284	.594
Liquid outflow as % of total outflow	84 5	83 4	83 3	86 8	3 7	.755	.875	.535

outflow via the rumen were due to differences in potassium outflow via the liquid phase. This resulted in lower apparent absorption of potassium from the rumen when energy was supplemented (P = .047) or acid infused into the rumen (P = .083). Giduck and Fontanot (1987) reported lower apparent absorption of potassium in wethers fed orchardgrass hay supplemented with energy. Giduck et al. (1988) observed that wethers infused intraruminally with HCl had decreased apparent potassium absorption measured by fecal and urine collections compared with wethers infused with water.

Liquid outflow of potassium as a percent of total outflow was greater than 99% and not different among treatments (Table 5-11). With all ruminal potassium in the liquid phase, it would be important to have a high concentration of sodium as well. This would help reduce the negative affects of potassium on magnesium absorption. Dietary intake of potassium was 4.6 times greater than sodium intake. Sodium is the major cation of saliva (Church, 1976). Saliva production may have been low due to the restricted feed intake of the animals and use of chopped hay in the ration. This could have resulted in a low Na:K ratio in the rumen which may have reduced apparent magnesium absorption (Martens and Blume, 1986). A factorial experiment needs to be conducted to determine if there is an interaction between Na:K ratio in the rumen and energy concentration of the diet on magnesium absorption by dairy cattle.

Total daily outflow of phosphorus from the rumen was increased by energy supplementation (P = .043) and acid infusion (P = .017) (Table 5-12). Outflow from the rumen of phosphorus in the liquid phase of the digesta was increased by energy supplementation (P = .027) or

TABLE 5-11. LEAST SQUARE MEANS FOR DAILY RUMINAL POTASSIUM OUTFLOW AND APPARENT ABSONPTION OF RUMINALLY-INFUSED HOLSTEIN CATTLE CONSUMING ALFALEA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

	Treatments					Contrasts		
Measurement	Control	Acid	Energy	Energy Buffer	SEM	Energy and Energy Buffer vs Others	Acid vs Control	Energy vs Energy Buffer
						P =		
Intake, g/d	158.0	159.2	159.6	159.7	2.0	.039	.329	.651
Liquid outflow, g/d	54.0	123.3	129.6	129.1	10.9	.029	.035	.975
Solids outflow, g/d	.3	.7	.5	.7	.17	.590	.299	.407
Total outflow, g/d	55.0	124.0	130.1	129.8	10.9	.029	.034	.985
Apparent absorption, %	63.1	24.2	15.2	10.0	8.9	.047	.083	.026
Liquid outflow as % of total outflow	99.4	99.4	99.6	99.4	.10	.526	.811	.435

TABLE 5-12. LEAST SQUARE MEANS FOR DAILY RUMINAL PHOSPHORUS OUTFLOW OF RUMINALLY INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

Measurement	Treatments				SEM	Contrasts		
	Control	Acid	Energy	Energy Buffer		Energy and Energy Buffer vs. Others	Acid vs. Control	Energy vs. Energy Buffer
						P =		
Intake, g/d	25.6	23.7	25.0	25.5	2.3	.206	.249	.915
Liquid outflow, g/d	4.4	11.1	11.6	10.6	.91	.027	.023	.549
Solids outflow, g/d	1.9	2.8	2.2	1.4	.40	.314	.360	.289
Total outflow, g/d	6.3	13.9	13.8	12.2	.93	.043	.017	.264
Liquid outflow as % of total outflow	72.7	81.3	83.9	88.0	3.9	.088	.280	.469

ecid infusion (P = .023). There were no treatment effects on phosphorus outflow from the solid phase. Energy supplementation tended to increase liquid phosphorus outflow as a percentage of total ruminal phosphorus outflow (P = .088). In a like manner, the amount of phosphorus soluble in ruminal fluid was increased by infusing an unbuffered versus a buffered VFA solution (Giduck et al., 1988). Phosphorus outflow from the liquid phase of the digesta as a percentage of total ruminal phosphorus outflow average 92%. This suggests that additional post-ruminal release of phosphorus from the solid phase could increase the supply of phosphorus to the intestine but the rumen was the major site of phosphorus release in feedstuffs.

Others have reported that energy supplementation did not affect apparent absorption of phosphorus from the digestive tract (Giduck and Fontenot, 1987). Harmon and Britten (1973) reported that increased energy intake by sheep increased both phosphorus and magnesium retention. A confounding factor might be saliva production. Saliva production would be higher by animals receiving E and ES because DMI was greater. Grace et al. (1974) reported a net secretion of phosphorus into the rumen due to saliva of 70 to 120% of dietary intake in sheep fed ryegrass. The observed increase in ruminal phosphorus outflow may have been a result of increased salivary phosphorus inflow.

Energy supplementation increased total copper outflow from the rumen (P = .044) (Table 5-13). This increase in copper outflow could be explained by differences in outflow of copper from the liquid phase of the digesta. Ruminal copper outflow from the liquid phase was increased 7.8 mg/d (P = .046) when energy was supplemented in the diet. Most of

TABLE 5-13. LEAST SQUARE MEANS FOR DAILY RUMINAL COPPER OUTFLOW OF RUMINALLY INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

Measurement	Treatments					Contrasts		
	Control	Acid	Energy	Energy Buffer	SEM	Energy and Energy Buffer vs Others	Acid vs Control	Energy vs Energy Buffer
						P =		
Intake, mg/d	40.4	40.0	47.6	47.5	.19	.031	.329	.651
Liquid outflow, mg/d	15.0	16.7	21.5	25.8	2.5	.046	.728	.293
Solids outflow, mg/d	6.4	16.5	14.8	11.6	3.3	.607	.168	.500
Total outflow, mg/d	21.4	33.1	36.3	37.3	3.0	.044	.109	.826
Liquid outflow as % of total outflow	65.3	53.5	58.6	69.7	6.5	.492	.367	.289

this increase could be accounted for by a 7 3 mg/d difference in copper intake between E plus E0 and C plus A. Acid infusion tended to increase total ruminal copper outflow (P = .109).

Liquid outflow of copper from the rumen as a percent of total ruminal outflow was not different among treatments. Liquid outflow of copper averaged 62% of the total ruminal copper outflow. This suggests that post-ruminal release of copper from the solid phase of the digesta could contribute significantly to the flow of copper reaching the small intestine.

Total ruminal outflow of zinc tended to be increased by energy supplementation (P = .073) and ruminal acid infusion (P = .093) (Table 5-14). Zinc outflow from the rumen was increased 55 mg/d by energy supplementation and 50 mg/d by acid infusion. Zinc outflow with the solid phase was not affected by treatment. Differences observed in total ruminal zinc outflow could be partially accounted for by differences in liquid outflow of zinc. Energy supplementation increased zinc outflow from the liquid phase of the ruminal digesta 21 mg/d (P = .064). Acid infusion increased zinc outflow from the liquid phase of the digesta 14 mg/d. This was not significant due to a large SEM. There are two factors which make interpretation of the results difficult. First, daily intake of dietary zinc was 77 mg more by animals on E and E0 than those on C and A (P = .001). Second, a large SEM (13) and too few degrees of freedom (1 and 3 for the F statistic) made it impossible to detect differences in zinc outflow with the solid phase of the digesta. It is possible that the effect of energy

TABLE 5-14. LEAST SQUARE MEANS FOR DAILY RUMINAL ZINC OUTFLOW OF RUMINALLY INFUSED HOLSTEIN CATTLE CONSUMING ALFALFA HAY WITH OR WITHOUT SUPPLEMENTAL ENERGY

Measurement	Treatments				SEM	Contrasts		
	Control	Acid	Energy	Energy Buffer		Energy and Energy Buffer vs Others	Acid vs Control	Energy vs Energy Buffer
						P =		
Intake, mg/d	107.1	102.3	192.4	190.8	2.9	.001	.329	.851
Liquid outflow, mg/d	70.1	83.9	96.2	110.1	9.7	.064	.467	.358
Solids outflow, mg/d	30.6	66.8	60.8	45.7	13.0	.735	.202	.444
Total outflow, mg/d	160.7	150.7	157.0	155.8	12.1	.073	.093	.948
Liquid outflow as % of total outflow	66.0	58.7	60.5	71.2	8.4	.600	.551	.294

supplementation on total zinc outflow from the rumen was due to differences in dietary zinc intake.

In conclusion, energy supplementation of an alfalfa diet reduced ruminal pH. Total millimoles of VFA was increased and the molar percentage of acetic acid decreased. Molar percentage of propionic acid tended to increase with energy supplementation. The result was a decrease in the acetate to propionate ratio compared with unsupplemented diets. Intraruminal acid infusion did not change ruminal pH or VFA concentrations. Buffering of an energy supplemented diet with sodium bicarbonate increased ruminal pH, total millimoles of VFA and the molar percentage of acetic acid but did not result in an increase in acetate to propionate ratio compared with unbuffered diets.

Energy supplementation and acid infusion decreased in situ DM disappearance of alfalfa hay. Disappearance of NDF was not different among the treatments. Phosphorus release was increased by acid infusion compared to the control treatment. In situ release of all other minerals was not affected by dietary treatments.

Energy supplementation of an alfalfa hay diet increased the ruminal concentrations of calcium in the liquid phase of the digesta and decreased calcium concentration in the solid phase. This resulted in greater ruminal calcium outflow in the liquid phase. A greater proportion of calcium in the liquid phase could increase supply of calcium postruminally available for absorption. Energy supplementation also increased total ruminal phosphorus outflow and liquid phosphorus outflow as a percent of total phosphorus outflow compared with unsupplemented diets. This could potentially increase the supply of

phosphorus reaching the duodenum and thereby increase phosphorus absorption. Energy supplementation decreased magnesium concentration in the solid phase of the ruminal digesta but did not result in increased magnesium outflow from the rumen. Greater than 83% of the ruminal magnesium outflow and 99% of ruminal potassium outflow was in the liquid phase. This suggests that potassium could interfere with magnesium absorption from the rumen. Both energy supplementation and acid infusion increased potassium outflow from the rumen. This could possibly reduce postruminal magnesium absorption if ruminal sodium outflow was low.

Treatment effects on movement of copper and zinc in the ruminal digesta were difficult to separate from the effect of dietary intake. For both of these minerals, energy supplementation was confounded with substantial increases in dietary intake above unsupplemented diets, 20% for copper and 80% for zinc (Table 5-2). Ruminal liquid outflow of copper as a percent of total ruminal copper outflow was 54 to 70%. This suggests 30 to 46% additional copper could be released from the solid phase of the digesta passing through the abomasum. Ruminal liquid outflow of zinc as a percent of total ruminal zinc outflow was 59 to 71%. This suggests 29 to 41% additional zinc could be released from the solid phase of the digesta passing through the abomasum.

Potassium was the major cation in the ruminal liquid phase of the minerals studied. Calcium was the major cation in the solid phase of the digesta. Copper concentration in the rumen was low, less than 1 ppm. This suggests that copper may be forming insoluble complexes with other dietary components or being sequestered by ruminal

microorganisms. Additional research should examine the movement of copper in the different phases of the digesta after it leaves the rumen. Zinc concentration in the liquid phase was very low compared to dietary concentration. Clearly, postruminal release of zinc from the solid phase of the digesta must occur if zinc is to be available to the animal. Additional research should examine the movement of zinc from feedstuffs and inorganic sources in the digesta postruminally.

This experiment had hoped to examine the effect of low ruminal pH and energy concentration of the diet on fiber disappearance, mineral release and movement of minerals within different ruminal digesta pools. For most of the measurements, the combination of high energy and low pH (E) did not differ from high energy and high pH (EH) dietary treatments. Yet differences in response did occur between the low energy and high pH (C) treatment and the low energy and low pH treatment (A). This suggests that an interaction exists between energy concentration and ruminal pH. This experiment was not a factorial design so interaction effects could not be tested. Future experiments should be designed as factorial experiments to determine if such an interaction does exist between ruminal pH and energy concentration of the diet.

CHAPTER VI
SUMMARY AND CONCLUSIONS

In experiment one, the in situ technique was able to differentiate among forage species for the proportion of total mineral found in the rapidly released, slowly released and unreleased mineral fractions. Ruminal release of all minerals from the six forage species was high. Amount of rapidly released mineral differed among forage species. Ranking of minerals according to amount rapidly released was potassium > magnesium > copper > phosphorus > calcium > zinc. Greater than 98% of the total potassium in all six forage species was rapidly released in the rumen. Percentage of the total magnesium rapidly released ranged from 72 to 92% for the six forage species. Amount of rapidly released calcium varied among the six forage species, ranging from -20% for E to 53% for BA. Calcium from A did not appear to be potentially less available compared to the other forage species. Alfalfa had the second highest coefficient for rapid release, 47%, among the six forage species. The quantity of phosphorus rapidly released from the six forage species had a range of 46% for P to 84% for E. Amount of copper rapidly released from the six forage species was variable, but 51 to 85% of the total copper was in this fraction. Zinc had the lowest coefficient for immediate release. Amount of zinc rapidly released was 7 to 43% among the six forage species. Both zinc and calcium had a larger slowly released fraction than the other minerals.

Extent of ruminal mineral release varied with the forage species and mineral. Minerals consistently split into three groups; potassium and magnesium had highest release coefficients; the middle group, phosphorus and copper, and the third group, calcium and zinc with the lowest ruminal release. In experiment one, extent of ruminal release of minerals from the six forage species was ranked as potassium > magnesium > phosphorus > copper > zinc > calcium. The most variable of the minerals was calcium. Ruminal release of calcium from A was 59, 74 or 86% in the three experiments. In the first experiment, it appeared that calcium in A was less available than calcium in other forage species. This was not the case in experiment two, where calcium from A was the most available. Bermudagrass, BA, and L had lower extent of ruminal release for phosphorus, copper and zinc compared with A, P, and E. Even though these release coefficients for BE, BA, and L were lower than those for A, P, and E, they were still high. Release coefficients for BE, BA, and L were 65 to 76% for calcium, 88 to 90% for magnesium, 64 to 81% for phosphorus, 70 to 82% for copper, and 53 to 67% for zinc. Because of this, it does not appear that NDF concentration in these forage species at this stage of maturity severely reduced mineral release.

The rumen was the major site of release for all six minerals. Acid-pepsin incubation increased calcium release from all forage species. Phosphorus was the only macromineral that had larger release coefficients in the intestines than in acid-pepsin solution or the rumen. The concentration of calcium and magnesium of BA, and L increased when passing through the small intestine. Bermudagrass

increased in calcium concentration when passing down the lower gut The result of experiments one and two suggest that forage species was a significant factor affecting rate and extent of mineral release In experiment two, there was a significant interaction between forage species (F) and digestion site (S) for DM, calcium, magnesium and phosphorus release In both experiments, cow (C) variability was a significant source of variation affecting mineral release A (F) by (C) interaction was significant for calcium, magnesium, and phosphorus release from the six forage species Because of these interactions (F by S, and F by C), one should be cautious about making generalized statements concerning mineral release from forages

In experiment three, energy supplementation of an alfalfa hay diet increased the total millimoles of VFA and decreased ruminal pH Lowering ruminel pH by energy supplementation or acid infusion reduced in situ DM disappearance Mineral release was not reduced by lower ruminal pH compared with control. The major effect of energy supplementation or acid infusion was on movement of minerals between the liquid and solid phase of the digesta and on ruminal mineral outflow Decreasing ruminal pH by energy supplementation or acid infusion increased the calcium concentration in the liquid phase of the ruminal digesta This resulted in a greater portion of total ruminal calcium outflow being in the liquid phase Energy supplementation or acid infusion increased ruminal liquid outflow of phosphorus This resulted in a greater proportion of total ruminal phosphorus outflow being in the liquid phase of the digesta For both calcium and phosphorus greater

ruminal liquid outflow could increase the supply of potentially available calcium and phosphorus to the duodenum

Potassium was the major cation in the liquid phase of the digesta. Calcium was the major cation in the solid phase of the digesta. Apparent ruminal magnesium absorption was not altered by energy supplementation or acid infusion compared with control diet. Apparent ruminal potassium absorption was decreased by energy supplementation or acid infusion. This was evident in the increased ruminal potassium outflow on these treatments compared with control. Because animals on the energy and energy buffered treatments responded similarly and animals on the control and acid treatments responded differently for certain measured variables, it appears that an interaction between energy and ruminal pH exists for those variables. This should be investigated further using a factorial experimental design.

In conclusion, the hypothesis that forage species with high NDF concentrations have reduced mineral availability compared to forage species with lower NDF concentration is not true consistently. The data from these three experiments indicate that forages with high NDF concentrations can have high mineral release. Certain forages have the ability to increase in calcium and/or magnesium concentration as they pass through the digestive tract. It is clear that forage species are different. These differences could influence the acid-base balance in the digestive tract. This is based on the following observations:

1. Almost 100% of the potassium in all six forage species is rapidly released in the rumen

2. Release of magnesium from all six forages was high, generally greater than 80%. This release occurred within 24 h in the rumen.

3. Release of calcium, phosphorus, copper and zinc varied with forage species and ruminal environment.

4. The major cations influencing ruminal osmotic pressure and pH would be sodium, potassium, calcium, and magnesium. Potassium, calcium, and magnesium concentrations in the liquid phase appear related to dietary intake.

5. The cell wall matrix of certain forages could reduce the amount of soluble calcium, copper and zinc in the digestive tract. This is based on the low concentration of copper and zinc in the liquid phase even though release from forages was high. Copper and zinc could be forming insoluble complexes, binding to forage cell wall, or taken up by rumen microbes.

6. Ruminal pH and energy concentration alter the concentration of minerals in both the liquid and solid phase of the ruminal digesta. Daily ruminal outflow of the minerals was altered to a greater extent by changes in liquid outflow compared to mineral outflow from the solid phase.

Given these observations, additional research is needed in the following areas.

1. Chloride content and release of chloride from forages in the digestive tract.

2 Effect of forage species on acid-base balance in the digestive tract

3 Effect of stage of maturity on mineral release from forages

4 Effect of ruminal environment on the concentration of minerals in the liquid and solid phase of ruminal digesta as effected by forage species

5. Sodium and potassium content of the rumen fluid as influenced by forage species and its effect on magnesium absorption from the rumen

APPENDIX
PROBLEMS AND DIFFICULTIES ENCOUNTERED DURING RUMINAL INFUSION OF STARCH AND HCl SOLUTIONS

To study the effect of readily fermentable carbohydrates and ruminal pH on ruminal mineral release and fiber disappearance from alfalfa hay required the infusion of starch and HCl into the rumen. The following problems occurred during this experiment as described in chapter V.

1. Ruminal infusion of starch was not possible as the starch solution (2500 g in 4 L distilled water) would clog the tubing used for the infusion. Starch would not stay in suspension unless the suspension was stirred constantly using a stir plate. During the infusion the starch would precipitate out of the suspension and form a hard mass in the tubing. This would cause blockage of the tubing. Because of this, the starch was mixed with the chopped hay and fed to the animals. The animals would not eat the hay and starch combination. They may have consumed this mixture if molasses could be mixed into the diet. This was not possible as molasses would have provided additional minerals in the diet. After this attempt to feed the starch failed, the starch was placed directly into the rumen and mixed into the digesta. This was not successful as the starch precipitated out of the liquid phase and formed a ball shaped mass of starch in the bottom of the ventral rumen sac. This mass of starch was

not digested rapidly as ruminal pH did not decrease below 6.2. Because of these difficulties, starch was replaced by ground corn in the diet. It may be possible to infuse starch into the rumen if more powerful pumps are used. The multistatic pumps used for this trial (chapter V) had to be set at their maximum speed of 10 to achieve the desired infusion rate. Infusion of starch using a gravity flow method with larger tubing would be possible if infusion rate was rapid as to prevent the starch from precipitating from the suspension.

2. Infusion of .36 N HCl did not decrease ruminal pH of these animals below 6.3. Because of this, the acid concentration of the infusion solution was increased to 1 N HCl. Placement of the infusion tubing was critical. It appeared that if the infusate came in close contact with the rumen lining ulcerations developed. This occurred in two of the four animals and caused them to decrease DMI. It appeared that the tubing needed to be placed in the center of the ventral rumen sac to prevent the problems described previously. This was difficult as the tubing would move with the flow of the ruminal digesta. A heavier and thicker tubing may prevent this problem.

3. Bloat occurred in three of four animals consuming rhizoma peanut hay. This was the forage of choice for this trial but had to be replaced with alfalfa hay because of the bloat and decreased DMI by animals. The cause of the bloat and decreased DMI may have been the particle size of the chopped rhizoma peanut hay. Based upon visual observation of ruminal contractions, rumination was decreased when animals consumed chopped rhizoma peanut hay compared with chopped alfalfa hay. The rhizoma peanut hay had finer stems and a greater proportion of leaves compared to the

alfalfe hay. The chopped rhizome peanut appeared to have a smaller particle size compared to the chopped elfelfa hay. When enimels were switched from chopped rhizome peanut hay to chopped alfalfa hay, DMI improved end the bloated condltions in the rumen subsided in ebout one week.

4. Uree was originally in the infusetes to balance the dietery treatments for nitrogen. It was removed from the infusates because animals appeared to have depressed DMI when urea was included in the contrel and acid dietary treatments. Energy supplementetion (E and EB) helped maintain DMI when infusetes contained urea. This suggests that possibly those animals receiving the control and ecid treatments were not able to utilize the soluble urea. Once urea was removed from these infusates DMI increased and was more consistent on e delly besis. A secondary facter could be the large quantity of ammonium chloride in the cuntrol infusate. It would be possible to avoid these preblems if KCl was the chloride source rather than ammonium chloride. This would reduce the quentity of rapidly soluble nitrogen in these treetments.

These are the major difficulties encountered during this trial. This trial should be repeated and it is hoped that these observations will help avoid some problems for the next research sclentist.

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BIOGRAPHICAL SKETCH

Stephen Mark Emanuele was born on April 9, 1955. He attended A. G. Berner High School in Massapequa, N. Y. Stephen attended the University of Rhode Island, obtaining a B.S. degree in animal science in 1977. He completed graduate study in dairy science at the University of Maryland, receiving a M.S. degree in 1979. His program of study emphasized fundamental metabolism of proteins, energy minerals, and vitamins. His masters thesis was titled "The Effect of Protein Solubility and Limestone Buffer on Nitrogen Metabolism in Lactating Dairy Cows."

From 1980-1982, Stephen managed a small dairy and conducted extension programs for Sudan Interior Mission in Maradi, Niger Republic, West Africa. Returning to the U.S. with his family, Stephen was an Assistant County Agent for Louisiana State University in Lake Charles, Louisiana from 1982-1985. He was responsible for 4-H livestock programs in dairy, sheep, swine, goats, rabbits, and chickens projects.

In January 1985, Stephen entered the University of Florida, where he will obtain a Doctor of Philosophy degree in Animal Science. Stephen is currently a member of Phi Kappa Phi and Gamma Sigma Delta honor societies, American Society of Animal Science, American Dairy Science Assoc., American Forage and Grassland Council, and Florida Farm Bureau.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

David K. Beede, Chairman
Associate Professor of Dairy Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Charles R. Staples, Cochairman
Assistant Professor of Dairy Science

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Clarence B. Ammerman
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy.

Joseph H. Conrad
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

John E. Moore
Professor of Animal Science

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

Otto C Ruelke
Professor of Agronomy

This dissertation was submitted to the Graduate Faculty of the College of Agriculture and the the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy

May, 1989

Dean, College of Agriculture

Dean, Gradnate School